3-1-2018

The Tensile Root Strength of Emergent Coastal Macrophytes

Lauris Olivia Hollis
Louisiana State University and Agricultural and Mechanical College, lholli8@lsu.edu

THE TENSILE ROOT STRENGTH OF EMERGENT COASTAL MACROPHYTES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by
Lauris Olivia Hollis
B.A.A.S, Stephen F. Austin State University, 2010
M.S. Stephen F. Austin State University, 2013
May 2018

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. R. Eugene Turner, and my de facto co-advisor, Dr. Nancy Rabalais, for their tremendous support during my dissertation research. I would like to acknowledge my committee members Drs. Sibel Bargu-Ates, Kanchan Maiti, Tracy Quirk, and Jim Wilkins for their time and input on my dissertation. Special thanks to Dr. Edward Laws for serving as the graduate school representative on my committee and for his keen insight on all matters statistical. I want to also thank the Louisiana State University Board of Regents and the Royal Dutch Shell Corporation for their generous research fellowships. I am grateful to the Coastal Ecology Section research associates namely Jim Lee, Charles Milan, Gary Peterson, Tom Aepelbacher, and especially Erick Swenson, as well as post-doctoral researchers, Drs. Charlie Martin , Jill Olin, and Rachael Blake.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Spartina patens is a dominant emergent macrophyte in fresh, intermediate, and brackish marshes along the Atlantic and Gulf coasts of United States where its biomechanical properties are a key component of wetland health and resilience. Its root biomass and tensile root strength are essential for anchorage, erosion protection, and are important determinants of soil strength. Nutrients and the herbicide atrazine are suspected of negatively impacting this wetland plant and others. The objectives of this study were to: 1) ascertain the tensile root strength of five emergent coastal macrophytes in coastal estuaries, and 2) test the effects of nutrient addition, atrazine exposure, flood duration, and possible interactive effects of these natural and anthropogenic stressors on the tensile root strength of *S. patens.* The tensile root strength of five coastal macrophytes declined with depth in four estuaries in southeastern Louisiana. The tensile strength of *Panicum hemitomon* and *Sagittaria lancifolia* growing in a wastewater treatment wetland also declined relative to a reference wetland site. The results from multiple greenhouse experiments demonstrated that combinations of nitrogen, phosphorus, and nitrogen+phosphorus resulted in the loss of about 50% tensile root strength of *S. patens* after four months. Atrazine treatments resulted in similar tensile root strength losses. The belowground biomass declined with nutrient and atrazine additions and in combination. The tensile root strength of *S. patens* varied depending on soil texture and flood duration regimes. The formation of aerenchyma tissue in response to flooding and the cessation of nutrient foraging by roots were the main factors that contributed to lower tensile root strength and less belowground biomass production. The field survey and greenhouse experiments results indicated that prolonged exposure to ambient levels of nutrient loads and atrazine weakens the tensile strength and degrades the belowground biomass. Prolonged inundation may exacerbate the effects of xenobiotics. The long-term effects

of these multiple stressors may facilitate coastal land loss. Management efforts can ameliorate the effects of poor water quality on wetlands by amending agricultural practices and land use zoning.

CHAPTER 1
GENERAL INTRODUCTION

Coastal wetlands perform numerous ecological functions and provide a plethora of ecological services that enhance the sustainability of human societies. In particular, coastal marshes attenuate storm surge energy, export nutrients and organic matter to adjacent ecosystems, improve water quality, provide habitat for numerous species of wildlife, and serve as nursery habitat for commercially important marine and estuarine species. Coastal wetland soils provide the substrate for vegetation and serve as sources, sinks, or transformers of chemical compounds. Two classes of soils dominate coastal wetlands: mineral soils, which are comprised of inorganic parent material, and organic soils, which are formed by decomposed plant and organic materials. These highly valuable and productive systems are threatened by the effects of climate change, such as rising sea levels and the increased frequency of tropical cyclones, which may erode wetland soils. Coastal marshes can keep pace with rising seas via accretion of organic matter which contributes to the elevation of coastal wetland soils (Turner et al. 2009, Turner 2011). However, these systems may be compromised by the influx of nutrients containing electron acceptors that utilize carbon electron donors in oxidation-reduction reactions that reduce belowground biomass. A ubiquitous source of these nutrients is cultural eutrophication, which is discussed next.

CULTURAL EUTROPHICATION

The increased nutrient loading to wetlands from what is broadly described as cultural eutrophicaton, may have deleterious effects on coastal wetland ecosystems as a result of increased rates of denitrification and organic matter decomposition, reduced root and rhizome biomass, and a weakened soil (Wigand et al. 2009, Deegan et al. 2012, Morris et al. 2013, Wigand et al. 2014; Turner 2011). Valiela et al. (1976), for example, reported reduced rhizome

and root biomass production as a result of nitrogen and phosphorus additions to coastal marshes in Massachusetts, USA. Langley et al. (2009) reported that nitrogen additions to a tidal Chesapeake Bay marsh reduced belowground biomass production and negated the increase in root biomass that was stimulated by elevated atmospheric CO_2 concentration. Morris et al. (2013) stated that under anaerobic conditions, high nitrate loads could stimulate the decay of decay-resistant organic matter in peat marshes where organic matter is the dominant constituent of the soil volume. In the Jamaica Bay Estuary of Long Island, New York, Wigand et al. (2014) used computer-aided tomography (CT) imaging to document lower belowground biomass and organic matter accumulation in the deteriorating Big Egg and Black Bank *Spartina alterniflora* marshes that had been exposed to long-term, anthropogenic inputs of wastewater nutrients. Excessive nitrate loading in a New England salt marsh reduced the biomass of roots of *Spartina alterniflora,* a reduction that may have contributed to the structural failure of marsh creek banks (Deegan et al. 2012). Wigand et al. (2009) reported a positive relationship between soil respiration rate and nitrogen loading at the watershed scale and an inverse relationship between respiration and soil organic carbon. In addition, elevated carbon dioxide emissions, which are indicative of increased decomposition rates, were detected in marshes treated or exposed to high nitrogen loads (Wigand et al. 2009; Morris and Bradley 1999).

Other studies have suggested that excess phosphorus concentrations may contribute to the degradation of coastal marshes. Zhang et al. (2012), for example, documented the release of bioavailable phosphorus under anaerobic conditions that were created by the suspended sediment load brought into the Breton Sound Estuary marshes (southeast Louisiana) by way of the Caernarvon River Diversion. This influx of P-enriched runoff could have an adverse effect on coastal water quality as well as the belowground biomass of wetland macrophytes in coastal

marshes. For example, Darby and Turner (2008a) reported a reduction in belowground biomass with increased phosphorus availability in *Spartina alterniflora* salt marshes in the Mississippi Delta. They found that plant resource allocation to belowground biomass was reduced in all plots where phosphorus had been added alone, or in concert with nitrogen and iron. In addition, Darby and Turner (2008b) reported a reduction of live belowground biomass in salt marshes at 12 of 14 sites during fertilization experiments conducted in Nova Scotia, Massachusetts, Virginia, and Louisiana. They suggested that P-additions, both alone or in combination with nitrogen, reduced the total belowground biomass and reduced the root + rhizome to shoot ratio because of decreased root foraging for nutrients. Swarzenski et al. (2008) reported that the substrate of Penchant Basin freshwater marshes of south Louisiana was more reduced and the soil organic matter was more decomposed than in marshes without long-term influxes of alkaline, nutrient-rich river water. They suggested that the reduction of nitrate and sulfate causes organic matter mineralization, which increases the concentration of sulfide in the organic substrate. As a result, the increased concentrations of sulfide can mobilize phosphate by interfering with its binding to iron-hydroxides, an effect known as ‘‘internal eutrophication,’’ due to the increased nutrient concentration that occurs without the input of additional external sources (Swarzenski et al. 2008,). Similarly, Wigand et al. (2015) reported lower percentages of organic matter in phosphorus treated soils in the Goat Island marshes of the North Inlet Estuary of South Carolina. The results of these field-based nutrient addition experiments suggest that excess nutrient loading of coastal marshes may have an adverse effect on emergent macrophytes. However, surface water runoff may contain other potentially harmful constituents such as toxic chemicals, pesticides, pharmaceuticals, and herbicides. A notable herbicide with widespread use is atrazine.

ATRAZINE CONTAMINATION

Atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is an s-triazine herbicide that was first registered for use in the United States in 1959 (USEPA 1994) and is primarily used to control undesirable broadleaf plants and grasses in agricultural operations. Non-agricultural applications of atrazine include the control of weeds on golf courses, railroad rights-of-way, residential lawns, and highways (USEPA 1994). From 2000 to 2010, an annual average of 72 million kilograms of atrazine was used on 71 million acres in the United States for agricultural operations (NASS 2011, USEPA 2016). The heaviest use of atrazine is associated with corn, sorghum, and sugarcane production, and the states with the highest usage are Illinois, Iowa, Nebraska, Indiana, and Kansas. Atrazine is transported from the point of application into surface waters through surface runoff and field tile drainage (Buhler et al. 1993, Paterson and Schnoor 1992, Southwick et al. 1990).

Atrazine is the most frequently detected pesticide in the surface waters of the Mississippi River watershed of the United States (Welch et al. 2014). Goolsby et al. (1997), detected atrazine in 98% of surface water samples from 132 streams in the upper midwestern United States. Battaglin et al. (2000) also detected atrazine in 100% of 129 samples from 75 rivers and streams in the midwestern United States in 1998. During a major flood in 2011, atrazine was detected at 100% frequency by 13 water quality monitoring stations in the lower Mississippi River-Atchafalaya River subbasin (Welch et al. 2014). Atrazine is absorbed by plant roots and translocated through the xylem to the leaves and apical meristem, where chlorosis and death are caused by inhibition of photosynthesis due to blockage of the transport of electrons to Photosystem I (Cejudo-Espinoza et al. 2009, Donnelly et al. 1993). Davis et al. (1979) found that ^{14}C-atrazine levels in *S. alterniflora* roots dropped from 77.9% to less than 3% 2 to 30 days after

absorption of the herbicide. Atrazine is mobile and persistent in the environment because of its low soil sorption coefficients and long half-life in soil and water. The relatively high water solubility (in comparison with other organic compounds) and environmental persistence of the parent atrazine compound may explain its prevalence in surface waters (USEPA 2016). Atrazine sorption in the soil may be affected by several factors such as organic matter content, dissolved organic carbon concentration, clay content, clay mineralogy, atrazine concentration, duration of exposure to soil, soil temperature, the composition of the soil microbial community, and soil moisture (Laird and Koskinen 2008, Albright 2011, USEPA 2016). In addition, atrazine has a low potential for volatilization and bioaccumulation because of its high solubility in water, low octanol-water partition coefficient, low Henry's Law Constant, and low vapor pressure (Table 1.1, USEPA 2016). Atrazine has a half-life of 168 days due to photodegradation in an aqueous pH 7 buffer solution exposed to natural sunlight. The average half-life of atrazine in soil at the soil surface, however, is 45 days under natural light (USEPA 2016). This half-life can vary, however, depending on the soil. The average half-life of atrazine ranges from 130 to 146 days in aerobic mineral soils, 38 to 155 days in aerobic aquatic environments, 49 to 608 days in anaerobic aquatic environments, and 159 days in anaerobic soils (USEPA 1994, USEPA 2016).

Atrazine degradation occurs primarily by microbial metabolism (Murphy 2009), but the degradation rates vary due to soil properties, temperature, and the composition of microbial communities (Cessna 2008, Laird and Koskinen 2008, Mandelbaum et al. 2008). There are two major classes of degradation products of atrazine (Fig 1.1). The primary metabolites deethylatrazine (DEA), deisopropylatrazine (DIA), and diadealkylatrazine (DDA) are formed by the dealkylation of the amino groups (USEPA 2016). The secondary metabolites hydroxyatrazine (HA), deisopropylhydroxyatrazine (DIHA), and deethylhydroxyatrazine (DEHA) are formed as a

result of the substitution of a chlorine atom by a hydroxy group as a result of hydrolysis (USEPA 2016).

Table 1.1 Physical and chemical properties of atrazine (USEPA 2016)

Physical/Chemical Property	**Value**
Chemical Formula	$C_8H_{14}ClN_5$
Molecular Weight	215.69 g mol^{-1}
Physical State	Powder
Color	White
Melting Point	175–177°C
Water Solubility (20° C)	33 mg L^{-1}
Vapor Pressure (20°C)	3.0 x 10^{-7} Torr
Henry's Constant (calculated)	2.6 x 10^{-9} atm-m^3 mol^{-1}
K_{ow}	501.18

Atrazine hydrolysis can occur either through biotic, microbial processes (Mandelbaum et al. 2008), or by abiotic processes catalyzed by acidic sites on mineral soil surfaces and organic compounds such as humic and fulvic acids, organic acids, and phenols (Laird and Koskinen 2008, Cessna 2008, USEPA 2016). The primary and secondary atrazine metabolites—DEA, DDA, DIA, HA, DIHA, and DEHA—can be formed by aquatic photodegradation; the soil photodegradation metabolites include only DEA, DDA, and DIA. The mobility of atrazine metabolites in the soil can vary from low to high, and DEA, DDA, and DIA exhibit greater mobility than HA because of their lower soil:water and organic carbon:water partition coefficients (USEPA 2016).

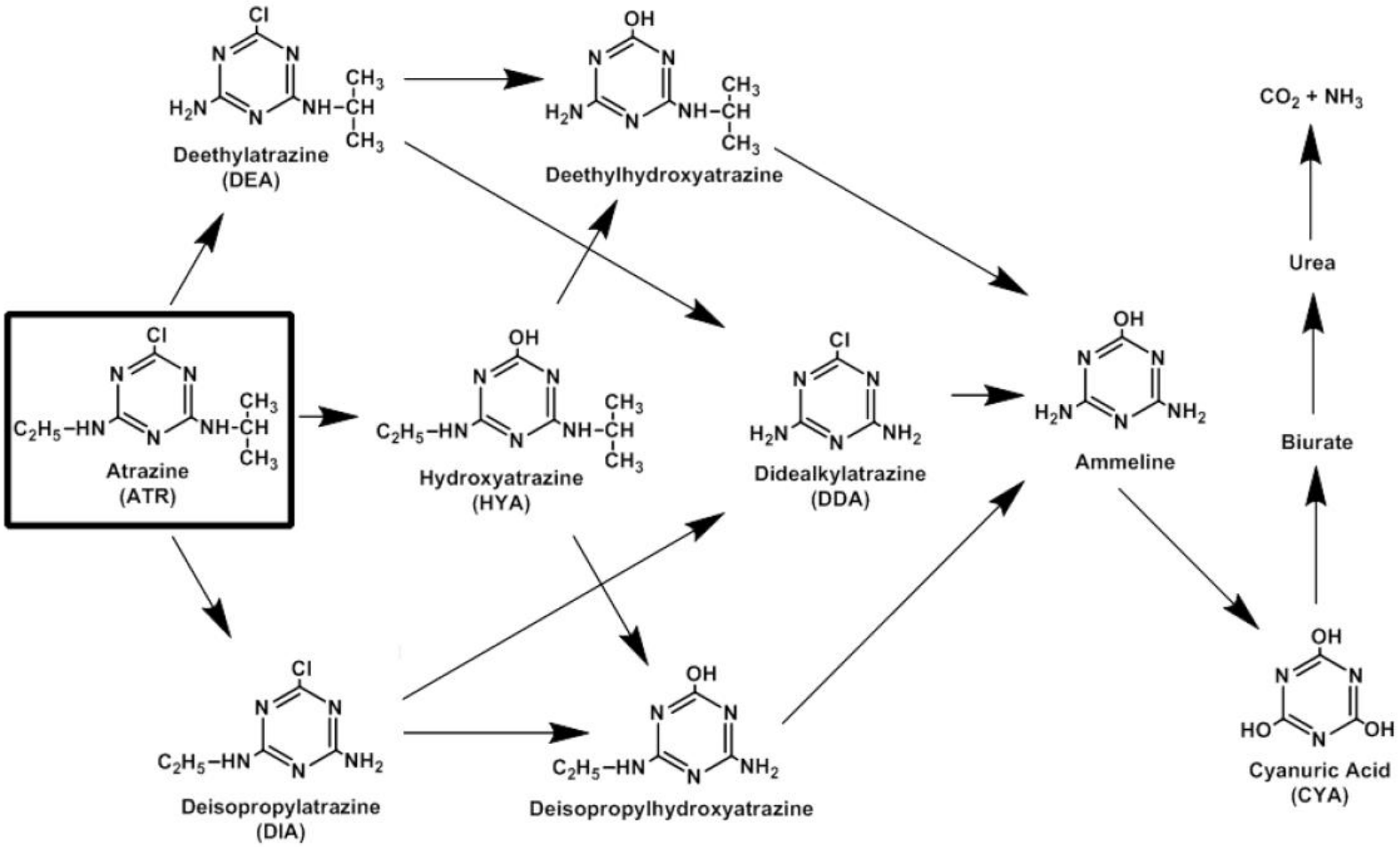


Fig. 1.1 Atrazine degradation pathways (reproduced from Albright and Coats 2012)

Atrazine has documented effects on nontarget organisms and ecosystems. For instance, photosynthesis in phytoplankton and periphyton populations is inhibited by atrazine concentrations ranging from 1 to 10 μg/L (Albright 2011), and Murphy (2009) has reported that the number of first-generation offspring of *Daphnia magna* is reduced after the adults are exposed to concentrations of atrazine greater than 250 μg/L. Atrazine may pose a threat to estuarine and nearshore marine ecosystems as well. Gao et al. (2011) found that *Zostera marina* seedlings exposed to 10 μg/L atrazine exhibited significantly lower plant fresh weight, lower total chlorophyll concentration, and up to 87% mortality at an atrazine concentration of 100 μg/L. Alvarez and Fuiman (2005) have reported that the larvae of the commercially important red drum (*Sciaenops ocellatus*) exposed to ambient levels of atrazine, exhibit a significantly reduced larval growth rate (7.9–9.8%). In addition, they have observed that atrazine-exposed larvae also exhibit significantly higher routine swimming speeds, swim in more convoluted paths, and are hyperactive. Other studies have also documented negative effects of atrazine on wetland plants. For instance, Bouldin et al. (2005) has observed that repeated exposure of macrophyte communities to atrazine can result in dieback of both atrazine-sensitive vegetation and tolerant monocotyledonous communities. Bouldin et al. (2006) have noted a lack of new root tissue emergence in *Juncus effusus* after 8 days of exposure to a field dose of atrazine (2.23 kg ha^{-1}). Langan and Hoagland (1996) have demonstrated in a microcosm experiment that atrazine can inhibit the growth of wetland plants at concentrations of 500 and 1500 $\mu g \cdot L^{-1}$. Consequently, atrazine contamination may threaten coastal wetland ecosystems by degrading the belowground biomass of emergent macrophytes.

SOIL TEXTURE

The tensile root strength of a plant may be greatly affected by a combination of soil texture and root architecture attributes. Soil texture is the proportion of sand, silt, clay, and organic matter in the soil and can affect the strength of a plant's anchorage in the soil. For instance, the grain sizes of soil components directly influence the formation of macro and micropores in the soil. Coarse soil textures, such as sand and organic materials, are much larger than fine-grained materials such as silt and clay. Consequently, coarse soil textures tend to create macropores and hold less water, whereas fine soil textures tend to create micropores, which generally hold more water due to adhesion of water molecules to soil clay minerals. These soil pores influence water flow, drainage, and water-holding capacity. The presence of saturated soil conditions alters redox potential and facilitates numerous biogeochemical reactions that have significant implications for wetland ecosystems, such as nutrient cycling, microbial community composition, and the mitigation of harmful effects of phytotoxins and xenobiotics. In turn, the root architecture of the plant can affect the texture and biogeochemical properties of the soil. Fibrous root systems, which are generally present in grasses (family *Poaceae*), create numerous conduits into the soil profile that can facilitate the percolation of surface water and oxygen diffusion. Also, fine roots and root hairs either senesce and become part of the exudates that are shed by the plant, or they are physically dislodged by root growth. This process creates organic deposits in the soil, which may serve as electron donors in redox reactions or become a binding mechanism for xenobiotics.

IMPORTANCE OF BELOWGROUND BIOMASS

Plant roots not only nourish the plant but also anchor it to the soil. Live roots of emergent plants take up nutrients and water as well as reinforce the stability of organic soils, while dead

roots eventually decompose and add refractory organic material to the substrate, which may contribute to the vertical accretion of coastal marshes. As a result, the proportions of live and dead roots in the belowground biomass may be an indicator of coastal marsh health. Phenotypic plasticity is the capacity of a genotype to express different phenotypes in different environments (Bradshaw 1965). Wetland plants exhibit anatomical, morphological, and physiological adaptations that allow them to persist in hypoxic and anoxic environments. One of the most frequent plastic responses of wetland plants to flooding is the formation of aerenchyma in the root cortex (Striker et al. 2006, Striker et al. 2007, Lamberti-Raverot and Puijalon 2012). Inundated or saturated emergent wetland macrophytes facilitate gas exchange under anaerobic conditions via oxygen transport through a series of interconnected lacunae (pore spaces) from aerial shoots to the roots, which allow the plant to cope with oxygen stress (Cronk and Fennessy 2001). However, the formation of aerenchyma in the root cortex causes a modification of the internal structure of the roots, which can lead to a trade-off among root mechanical strength properties (Striker et al. 2007). The strength and stability of the root mass may directly contribute to the strength and stability of organic marsh soils (Turner 2011, Turner et al. 2009). I measured the tensile root strength of several dominant wetland plants in the field and laboratory to investigate how exposure to nutrients and atrazine affected them. The tensile root strength concept and how it is measured is discussed in the next section.

TENSILE ROOT STRENGTH

Tensile strength is a biomechanical property of vegetation defined herein as the resistance of a material under tension to breaking. The tensile strength of vegetation describes a plant's resistance to uplifting forces; it is important because it reflects the ability of the plant to resist forces that cause uprooting from the soil. The biomechanical properties of a plant are driven

largely by the attributes of its constituent materials. For instance, elastic materials can undergo deformation and return to their original configuration once the load is removed. Plastic materials, on the other hand, deform under a load and do not recover their original configuration.

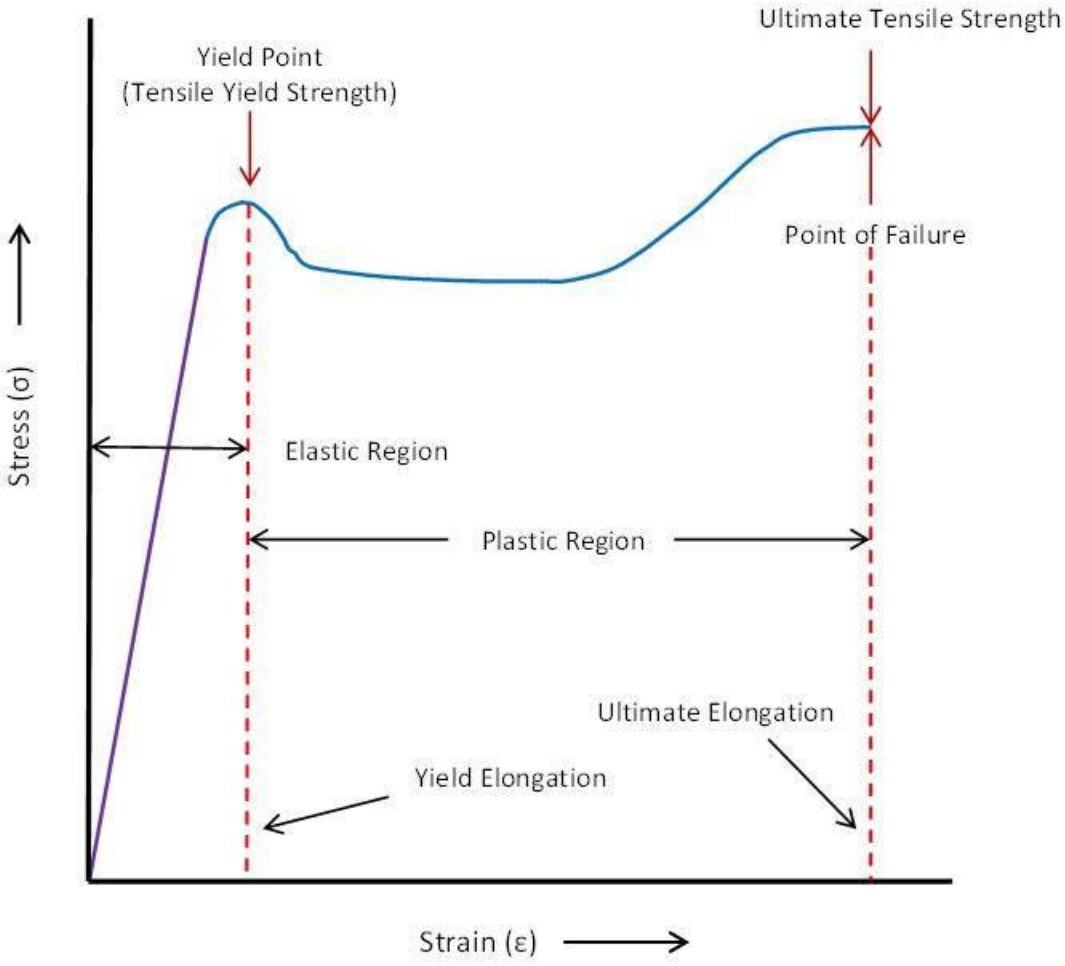


Fig. 1.2 Stress-strain diagram of uniaxial tensile forces (reproduced from Niklas 1992)

Most plants contain plastic materials. As a result, it is important to distinguish between tensile strength and tensile stress. Tensile strength may be the breaking force that occurs at the elastic or plastic limit of a material; whereas, the tensile stress is the breaking force per unit area of the material (Fig 1.2). In addition, tensile stress is the amount of force per unit area of a member,

where the area represents the original area, before deformation. Ultimate tensile stress, however, is the amount of force per unit of instantaneous area, which is the area of the member at failure that was subjected to deformation. The mechanical behavior of the cell wall infrastructure is dominated by the material properties of cellulose, which has a high tensile strength, a very high tensile modulus, and the capacity for considerable elastic extension in the direction of cellulose molecules (Niklas, 1992). The plant stem is able to resist tensile forces because of the cellulose in the cell walls and the turgor pressure within the stem (Niklas, 1992). The stem transfers load to the architecture of the root system, which acts as an anchor against pullout from the soil (Bouanchaud 2013). The ability of the roots to resist pullout forces is determined by the tensile capacity of the roots in conjunction with the frictional interface between the soil and the roots, which may depend upon several factors such as soil composition, soil properties, root composition, root density, root depth, and microbial activity (Teal et al. 2012, Niklas 1992, Niklas and Spatz 2012). Turgor pressure is another important biomechanical property because of its influence on the tensile stresses generated within cell walls and the mechanical stiffness of thin-walled cells and thin-walled tissues (Niklas, 1992). Turgidity (ψp) is defined as the difference between water potential (ψw) and solute potential (ψs), or $\psi p = \psi w - \psi s$ and refers to how fully protoplasts within cells are hydrated (Chatagnier 2012). Turgor pressure increases strength by placing cell walls in a state of axial tension. Ennos (1990) developed a function for pullout forces based on the strength of the bonds between the roots and soil:

$$FP = S \cdot L \cdot 2\pi r$$

where FP is the pullout force for an individual root (N), S is soil shear strength (kPa), r is the radius of the root (m) and L is the length of the root (m) (Pollen 2007). Therefore, soil shear strength may be directly related to the tensile strength of individual roots and rhizomes. Several

studies have documented a reduction in rooting depth as well as weak substrate structure and soil shear strength (Darby and Turner 2008a, Turner 2011, Howes et al. 2010, Turner et al. 2009). For instance, Swarzenski et al. (2008) found that the soil of an organic-rich freshwater marsh of maidencane (*Panicum hemitomon*) exposed to a chronic influx of nutrient-rich river water was more decomposed, and its strength was reduced by 50% compared with marsh soil substantively identical, except that nutrient-poor rain was the source of freshwater. Turner (2011) conducted shear vane soil strength testing in a Louisiana marsh and found that the soil strength was reduced under higher nutrient loading in fertilized plots versus control plots. Turner (2011) also tested canvas strips from these fertilized plots and recorded an 18–48% reduction in their tensile strength. These results suggest that the ability of coastal marshes to resist erosive forces such as storm surge and wave action may be diminished by factors that weaken the belowground biomass. The fibrous architecture of emergent macrophyte roots may function in a manner analogous to concrete reinforcement bars in organic soils and contribute to coastal marsh stability (Burdick 1989). Coastal marshes with organic soils keep pace with sea level rise by the vertical accretion of organic matter derived from decomposing plant material from the surface as well as from roots and rhizomes that add additional organic mass below the surface (Turner et al. 2004). Therefore, the health of the belowground biomass of the dominant emergent macrophytes will be a critical component in sustaining coastal wetland resilience in a changing global climate.

IMPORTANCE OF DOMINANT EMERGENT WETLAND MACROPHYTES

Several dominant wetland plants are included in this study. *Panicum hemitomon* Schultes is a clonal monocotyledonous grass found in freshwater-dominated areas along the coastal plain of the United States from New Jersey southward into Florida and westward along the Gulf Coast to Texas (Godfrey and Wooten 1979). *P. hemitomon* is the dominant emergent macrophyte of

coastal freshwater marshes in Louisiana, and it produces rhizome and root biomass that is crucial for thick-mat floating marsh structural integrity and buoyancy. (Chabreck 1972, Hester et al. 2001). *Spartina patens* (Ait.) Muhl. is a coastal grass species that is distributed in intermediate and brackish marshes along the Atlantic and Gulf coasts of North America from New Brunswick to south Texas (Godfrey and Wooten, 1979, Hester et al. 1996). In addition, it is the most ubiquitous emergent wetland species on the Louisiana coast (Chabreck 1972). *S. patens* exhibits variable ecological plasticity and its occurrence range extends from dunes and swales to coastal intermediate and brackish marshes, where it is frequently the dominant plant species (Chabreck 1972, Hester et al. 2001). *Spartina alterniflora* Loisel is an herbaceous, native, warm-season perennial grass that forms dense vegetative colonies along shorelines and inter-tidal flats (Darby and Turner 2008c). *S. alterniflora* commonly dominates coastal salt marshes along the Atlantic coast of North America from Newfoundland southward to Florida and westward along the Gulf Coast to Texas (Godfrey and Wooten, 1979). Fifty to ninety percent of the annual production of *S. alterniflora* in eastern US salt marshes occurs belowground as roots and rhizomes. This belowground biomass facilitates the accumulation of organic matter and maintains the vertical position of coastal marshes as sea level rises and marsh soils compact (Valiela et al. 1976, Smith et al. 1979, Pomeroy and Wiegart 1981, Giblin and Howarth 1984, Darby and Turner 2008c). *Sagittaria lancifolia* is a perennial herb that is a common wetland species of the Northern coast of the Gulf of Mexico and a major contributor to marsh building and aquatic food chains (Schussler and Longstreth 1996, Lindau and Delaune 1999). *S. lancifolia* may be found in marshes that range from fresh to intermediate salinity and can be a dominant species in some oligohaline marshes (Chabreck, 1972). *Schoenoplectus americanus* (Pers.) Volk. ex Schinz and Keller (formerly classified *as Scirpus olneyi*) is a rhizomatous, emergent, and perennial

macrophyte that often occurs in mixed stands in brackish marshes along the Gulf Coast with *S. patens* (Broome et al. 1995, Arreghini et al. 2006). Together, *S. patens, S. alterniflora, P. hemitomon, S. americanus,* and *S. lancifolia* comprise the bulk of emergent vegetation cover in over 1.4 million hectares of fresh, brackish, intermediate, and salt marshes on the Louisiana coast (Chabreck 1972).

The production of aboveground and belowground biomass of *S. patens, S. alterniflora,* and *P. hemitomon* directly contributes to the accretion of organic matter that comprises the organic soils in the majority of Louisiana coastal marshes. The biomass of these species maintains the structural integrity of those marshes (Hester et al. 1996, Hester et al. 2001, Darby and Turner 2008c). In addition, Mayence and Hester (2010) have stressed the importance of quantifying the relationship between root length, diameter, and tensile strength to the stability of floating freshwater marshes in coastal Louisiana. However, these species occupy areas that are frequently subjected to atrazine contamination and excess nutrient loads from the Mississippi River (Welch et al. 2014). Therefore, if the strength of their belowground biomass is compromised by these xenobiotics, then the stability of Louisiana coastal wetlands may be jeopardized.

The tensile strength of soils has been assessed using canvas strips (Maltby 1988, Turner 2011, Laursen 2004), but no study, to my knowledge, has tested the tensile strength of individual roots from five common wetland plant species in U.S. coastal wetlands that have been exposed to high nutrient loading or atrazine exposure. This study investigates whether excess nutrient loading, atrazine exposure, and natural abiotic factors such as soil texture and flood duration decreases the tensile root strength of emergent macrophytes in Louisiana coastal wetlands.

Chapter 2 reports on measurements of the tensile root strength of five of the most common emergent wetland macrophytes from Louisiana coastal marshes: *Spartina alterniflora, Spartina patens, Schoenoplectus americanus, Sagittaria lancifolia,* and *Panicum hemitomon*. Chapter 3 investigates the effects of atrazine exposure with different types of soil textures and their possible interactive effects on *S. patens*. Chapter 4 examines the effects of nutrient addition and atrazine exposure on the tensile root strength of *S. patens.* Chapter 5 explores the effects of flood duration and nutrient addition on the tensile strength of *S. patens* roots. Chapter 6 contains a multiple stressor experiment in which *S. patens* was subjected to various combinations of nutrient enrichment, atrazine exposure, and flood duration to ascertain the relative effects on tensile root strength. Ecological and management implications of these experiments are examined in Chapter 7, followed by a summary in Chapter 8.

REFERENCES

Albright III VC (2011) Investigations into the capabilities of switchgrass to phytoremediate atrazine contamination in surface water runoff. Master's thesis, Iowa State University, Ames, IA 58 pp.

Albright III VC, Coats JR (2012) Disposition of atrazine metabolites following uptake and degradation of atrazine in switchgrass. International Journal of Phytoremediation http://www.tandf.com/http://dx.doi.org/10.1080/15226514.2012.759528

Alvarez MC, Fuiman LA (2005) Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae. Aquatic Toxicology 74:229–241

Arreghini S, de Cabo L, Fabrizio de Iorio A (2006) Phytoremediation of two types of sediment contaminated with Zn by *Schoenoplectus americanus*. International Journal of Phytoremediation 8:223–232

Battaglin WA, Furlong ET, Burkhardt MR, Peter CJ (2000) Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the midwestern United States, 1998. Science of the Total Environment 248:123-133

Bouanchaud III JM (2013) The uplift resistance of *Spartina alterniflora* in a Louisiana salt marsh. Master's thesis, Louisiana State University, Baton Rouge, LA 47 pp.

Bouldin JL, Farris JL, Moore MT, Smith Jr. S, Stephens WW, Cooper CM (2005) Evaluated fate and effects of atrazine and lambda-cyhalothrin in vegetated and unvegetated microcosms. Environmental Toxicology 20:287-298

Bouldin JL, Farris JL, Moore MT, Smith Jr. S, Cooper CM (2006) Hydroponic uptake of atrazine and lambda-cyhalothrin in *Juncus effusus* and Ludwigia peploides. Chemosphere 65:1049-1057

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13:115-155

Broome SW, Mendelssohn IA, McKee KL (1995) Relative growth of *Spartina patens* (Ait.) Muhl. and *Scirpus olneyi* Gray occurring in a mixed stand as affected by salinity and flooding depth. Wetlands 15(1):20-30

Buhler DD, Randall GW, Koskinen WC, Wyse DL (1993) Atrazine and alachlor losses from subsurface tile drainage of a clay loam soil. Journal of Environmental Quality 22:583-588

Burdick DM (1989) Root aerenchyma development in Spartina patens in response to flooding. American Journal of Botany 76(5):777-780

Cejudo-Espinosa E, Ramos-Valdivia AC, Esparza-Garcia F, Moreno-Casasola P, Rodriguez-Vazquez R (2009) Short-term accumulation of atrazine by three plants from a wetland model system. Archives of Environmental Contamination and Toxicology 56:201-208

Cessna AJ, (2008) Nonbiological degradation of triazine herbicides: Photolysis and hydrolysis *In*: H Lebaron, J Macfarlane, O Burnside (eds.) The Triazine Herbicides: 50 years Revolutionizing Agriculture. Elsevier, San Diego, CA pp. 329-354

Chabreck RH (1972) Vegetation, water and soil characteristics of the Louisiana coastal region. Bulletin No. 664. Louisiana State University Press, Baton Rouge, LA 72 pp.

Chatagnier J (2012) The biomechanics of salt marsh vegetation applied to wave and surge attenuation. Master's thesis, Louisiana State University, Baton Rouge, LA 64 pp.

Cronk JK, Fennessy MS (2001) Wetland Plants: Biology and Ecology. CRC Press, New York, NY 462 pp.

Darby FA, Turner RE (2008a) Below- and aboveground biomass of *Spartina alterniflora*: Response to nutrient addition in a Louisiana salt marsh. Estuaries and Coasts 31:326–334

Darby FA, Turner RE (2008b) Effects of eutrophication on salt marsh root and rhizome biomass accumulation. Marine Ecology Progress Series 363:63–70

Darby FA, Turner RE (2008c) Below- and aboveground *Spartina alterniflora* production in a Louisiana salt marsh. Estuaries and Coasts 31:223–231

Davis DE, Weete JD, Pillai CGP, Plumley FG, and others (1979) Atrazine fate and effects in a salt marsh. United States Environmental Protection Agency Report EPA-600/3-79-111, 85 pp.

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM (2012) Nature 490:388-392

Donnelly PK, Entry JA, Crawford DL (1993) Degradation of atrazine and 2,4 dichlorophenoxyacetic acid by mycorrhizal fungi at three nitrogen concentrations In vitro. Applied and Environmental Microbiology 59(8):2642-2647

Ennos AR (1990) The anchorage of leek seedlings: The effect of root length and soil strength. Annals of Botany 65:409-416

Gao Y, Fang J, Zhang, J, Ren L, Mao Y, Li B, Du M (2011) The impact of the herbicide atrazine on growth and photosynthesis of seagrass, *Zostera marina* (L.), seedlings. Marine Pollution Bulletin 62(8):1628-1631

Giblin AE, Howarth RW (1984) Porewater evidence for a dynamic sedimentary ion cycle in salt marshes. Limnology and Oceanography 29:47-63

Godfrey RK, Wooten JW (1979) Aquatic and wetland plants of southeastern United States: Monocotyledons. The University of Georgia Press, Athens, GA 711 pp.

Goolsby D, Thurman EM, Pomes M, Meyer M, Battaglin W (1997) Herbicides and their metabolites in rainfall: Origin, transport, and deposition patterns across the midwestern and northeastern United States, 1990-1991. Environmental Science and Technology 31:1325-1333

Hester MW, Mendelssohn IA, McKee KL (1996) Intraspecific variation in salt tolerance and morphology in the coastal grass *Spartina patens* (Poaceae). American Journal of Botany 83(12): 1521-1527

Hester MW, Mendelssohn IA, McKee KL (2001) Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: Morphological and physiological constraints. Environmental and Experimental Botany 46: 277–297

Howes NC, FitzGerald DM, Hughes ZJ, Georgiou IY, Kulp MA, Miner MD, Smith JM, Barras, JA (2010) Hurricane induced failure of low salinity wetlands. Proceedings of the National Academy of Sciences (USA) 107:14014-14019 www.pnas.org/cgi/doi/10.1073/pnas.0914582107

Laird DA, Koskinen WC (2008) Triazine soil interactions *In*: H Lebaron, J Macfarlane, O Burnside (eds.) The Triazine Herbicides: 50 years Revolutionizing Agriculture. Elsevier, San Diego, CA pp. 275-300

Lamberti-Raverot B, Puijalon S (2012) Nutrient addition affects the mechanical resistance of aquatic plants. Journal of Experimental Botany 63(17):6115-6123

Langan MM, Hoagland KD (1996) Growth responses of *Typha latifolia* and *Scirpus acutus* to atrazine contamination. Bulletin of Environmental Contamination and Toxicology 57:307-314

Langley JA, McKee KL, Cahoon DR, Cherry JA, Megonigal JP (2009) Elevated CO_2 stimulates marsh elevation gain, counterbalancing sea-level rise. Proceedings of the National Academy of Sciences (USA) 106(15):6182-6186

Laursen KR (2004) The Effects of Nutrient Enrichment on the Decomposition of Belowground Organic Matter in a *Sagittaria lancifolia*-dominated Oligohaline Marsh. Master's thesis, Louisiana State University, Baton Rouge, LA 80 pp.

Lindau CW, Delaune RD (1999) Vegetative response of Sagittaria lancifolia to burning of applied crude oil. Water, Air, and Soil Pollution 121:161–172.

Maltby E. (1988) Use of cotton strip assay in wetland and upland environments – an international perspective. *In*: Harrison, A.F., Latter, P.M., Walton, D.W.H. (Eds.), Cotton Strip Assay: An Index of Decomposition in Soils. Institute of Terrestrial Ecology, Grange-Over-Sands, UK, pp. 140–154

Mandelbaum RT, Sadowsky MJ, Wackett LP (2008) Microbial degradation of s-Triazine herbicides *In:* HM LeBaron, JE McFarland, OC Burnside (eds.) The Triazine Herbicides: 50 years Revolutionizing Agriculture. Elsevier, San Diego, CA 601 pp.

Mayence CE, Hester MW (2010) Growth and allocation by a keystone wetland plant, *Panicum hemitomon*, and implications for managing and rehabilitating coastal freshwater marshes, Louisiana, USA. Wetlands Ecology and Management 18:149–163

Morris JT, Bradley PM (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. Limnology and Oceanography 44(3):699–702

Morris JT, Shaffer GP, Nyman JA (2013) Brinson Review: Perspectives on the influence of nutrients on the sustainability of coastal wetlands. Wetlands 33:975–988

Murphy IJ (2009) Uptake and degradation of atrazine utilizing phytoremediation technology with switchgrass (*Panicum virgatum*). Master's thesis, Iowa State University, Ames, IA 84 pp.

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago, IL 608 pp.

Niklas KJ, Spatz HC (2012) Plant Physics. University of Chicago Press, Chicago, IL 426 pp.

Paterson KG, Schnoor JL (1992) Fate of alachlor and atrazine in a riparian zone field site. Water Environment Research 64(3):274-283

Pollen N (2007) Temporal and spatial variability in root reinforcement of streambanks: Accounting for soil shear strength and moisture. Catena 69:197–205

Pomeroy LR, Wiegart RG (1981) Ecosystem and Population Ecology of a Salt Marsh: A Synthesis. Springer, Berlin, Germany 271 pp.

Schussler EE, Longstreth DJ (1996) Aerenchyma develops by cell lysis in roots and cell separation in leaf petioles in *Sagittaria lancifolia* (Alismataceae). American Journal of Botany 83(10):1266-1273

Smith K, Good RR, Good NF (1979) Production dynamics for above-belowground components of a New Jersey *Spartina alterniflora* tidal marsh. Estuarine Coastal Shelf Science 9:189-201

Southwick LM, Willis GH, Bengtson RL, Lormand TJ (1990) Atrazine and metolachlor in subsurface drain-water in Louisiana. Journal of Irrigation and Drainage Engineering 116:16-23

Striker GG, Insausti P, Grimoldi AA, Leon RJC (2006) Root strength and trampling tolerance in the grass *Paspalum dilatatum* and the dicot Lotus glaber in flooded soil. Functional Ecology 20:4–10

Striker GG, Insausti P, Grimoldi AA, Vega AS (2007) Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. Plant, Cell and Environment 30:580–589

Swarzenski CM, Doyle TW, Fry B, Hargis TG (2008) Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. Biogeochemistry 90:49–63

Teal JM, Best R, Caffrey J, Hopkinson CS, McKee KL, Morris JT, Newman S, Orem B (2012) Mississippi River freshwater diversions in southern Louisiana: Effects on wetland vegetation, soils, and elevation. *In*: AJ Lewitus, M Croom, T Davison, DM Kidwell, BA Kleiss, JW Pahl , CM Swarzenski (eds.) Final Report to the State of Louisiana and the U.S. Army Corps of Engineers through the Louisiana Coastal Area Science & Technology Program, National Oceanic and Atmospheric Administration 49 pp.

Turner RE, Swenson EM, Milan CS, Lee JM, Oswald TA (2004) Below-ground biomass in healthy and impaired salt marshes. Ecological Research 19:29–35

Turner RE, Howes BL, Teal JM, Milan CS, Swenson EM, Goehringer-Toner DD (2009) Salt marshes and eutrophication: An unsustainable outcome. Limnology and Oceanography 54(9): 1634-1642

Turner RE (2011) Beneath the salt marsh canopy: Loss of soil strength with increasing nutrient loads. Estuaries and Coasts 33:1084-1093. doi: 10.1007/s12237-010-9341-y

United States Department of Agriculture National Agricultural Statistics Service (NASS) (2011) Agricultural statistics: 2011. United States Government Printing Office, Washington D.C. 509 pp.

United States Environmental Protection Agency (U.S. EPA) (1994) Atrazine, simazine and cyanazine; notice of initiation of special review. Federal Register 59: 60412-60443

United States Environmental Protection Agency (USEPA) (2016) Refined Ecological Risk Assessment for Atrazine. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington D.C.

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: Belowground biomass. Limnology and Oceanography 21(2):245-252

Welch HL, Coupe RH, Aulenbach BT (2014) Concentrations and Transport of Suspended Sediment, Nutrients, and Pesticides in the Lower Mississippi-Atchafalaya River Subbasin During the 2011 Mississippi River Flood, April Through July. Scientific Investigations Report 2014–5100, 44 pp.

Wigand C, Brennan P, Stolt M, Holt M, Ryba S (2009) Soil respiration rates in coastal marshes subject to increasing watershed nitrogen loads in southern New England, USA. Wetlands 29(3):952–963

Wigand C, Roman CT, Davey E, Stolt M, Johnson R, Hanson A, Watson EB, Moran SB, Cahoon DR, Lynch JC, Rafferty P (2014) Below the disappearing marshes of an urban estuary: Historic nitrogen trends and soil structure. Ecological Applications 24(4):633–649

Wigand C, Roman CT, Davey E, Johnson R, Sundberg K, Morris J, Kenny P, Smith E, Holt M (2015) Nutrient effects on belowground organic matter in a minerogenic salt marsh, North Inlet, SC. Estuaries and Coasts DOI 10.1007/s12237-014-9937-8

Zhang W, White JR, DeLaune RD (2012) Diverted Mississippi River sediment as a potential phosphorus source affecting coastal Louisiana water quality. Journal of Freshwater Ecology 27(4):575–586

CHAPTER 2
THE TENSILE ROOT STRENGTH OF FIVE EMERGENT COASTAL MACROPHYTES

INTRODUCTION

A plant's ability to resist the erosive forces of waves, wind, herbivore grazing, gravity, and storm surge may be diminished by factors that weaken the belowground biomass. Waves, wind, herbivore grazing, and gravity can exert uprooting forces on plants. Higher nutrient loading in coastal wetland plants, for example, has been associated with lower live root and rhizome biomass (Darby and Turner 2008), faster organic matter decomposition (Wigand et al. 2009), and decreased soil shear strength (Turner 2011). Wetland erosion can occur if erosive forces exceed the ability of the belowground biomass to resist tensional and compressional loading. The erosion of wetland vegetation may lead to pond expansion, altered drainage patterns, dislodged or destroyed vegetation, shoreline erosion and marsh displacement. The impact of these destructive erosional forces is probably affected by the type, distribution, and health of wetland vegetation. The fibrous architecture of emergent macrophyte roots may function in a manner analogous to concrete reinforcement bars to support coastal marsh stability. Three examples from the agricultural literature are the following: 1) Fan and Su (2008) demonstrated how the roots of the annual legume, *Sesbania bispinosa* (Prickly Sesban), increased shear strength by 39 to 42% within soils in Kaohsiung City, Taiwan; 2) Ennos (1989) explored the mechanics of uprooting forces on seedlings of *Helianthus annuus* L. (Sunflower). He found that seedlings with longer roots (50 to 60 cm) required more force to extract them than those with shorter roots (10 cm); and 3) Comino and Druetta (2010) reported increases in soil shear strength and root displacement that exceeded 100% in Italian alpine soils that were reinforced by three grass species in the family *Poaceae*. The traits of individual roots, e.g.,

length, diameter, cross-sectional area, volume, sinuosity, age, and decomposition stage can affect the moisture content, bulk density, soil texture, shear strength, and organic matter content of soils. In addition, the morphological configuration of the belowground biomass of roots and rhizomes can contribute to the magnitude of soil reinforcement (diameter class distribution and depth distribution; as well as mechanical properties such as tensile root strength, tortuosity, elastic modulus, and root-soil friction) (DeBaets et al. 2008).

Soil shear strength can be calculated by measuring tensile strength, which is defined as the resistance of a material in tension to an external load (Niklas 1992, Wu et al. 1979). Tensile root strength data can be used to populate models and provide predictions of soil shear strength (Nyambane and Mwea 2011, De Baets et al. 2008, Wu et al. 1979). For example, Wu et al. (1979) devised a model of a soil-root system in which roots were placed in tension as a shearing force was applied to the soil:

$$s_r = t_r (\cos \theta \tan \Phi' + \sin \theta) \qquad (1)$$

where $\mathbf{s_r}$ is the shear strength of the soil due to roots (kPa), t_r is the total tensile strength of the roots per unit area of the soil (expressed as tensile stress in MPa m^{-2}). θ is the angle of shear distortion in the shear zone, and Φ' is the soil friction angle

For a range of θ from 48 to 72 degrees, the increase in soil shear strength (s_r) is:

$$s_r = 1.2t_r \qquad (2)$$

The shear strength of the soil-root system (s*) can be estimated by:

$$s^* = s + s_r \qquad (3)$$

where **s** is the soil shear strength.

Tensile root strength can be used to estimate the resilience and resistance of individual root masses to various erosive forces. These measurements, if repeated a sufficient number of times,

can be scaled up to provide a more accurate estimate of the strength of the soil-plant matrix. Both tensile and soil shear strength may be used to determine the impact of different potential stressors on the health of a wetland plant's belowground biomass and to predict areas that may be vulnerable to erosion and wetland loss. Many studies have investigated tensile root strength in regard to soil shear strength (DeBaets et al. 2008, Comino and Druetta 2010, Turner 2011, Nyambane and Mwea 2011, Muntohar 2012, Jain 2013), cotton fiber decomposition (Maltby 1988, Slocum et al. 2009, Baustian et al. 2010, Bodker et al. 2015,), soil stabilization (Wu et al. 1979, Genet et al. 2007, Pollen 2007, Comino et al. 2010, Yang et al. 2016), plant uprooting resistance (Ennos 1989, Ennos 1990, Easson et al. 1995, Mickovski et al. 2007, Osman et al. 2011, Crouzy et al. 2014), submerged aquatic plants (Puijalon et al. 2007, Puijalon et al. 2008, Lamberti-Raverot and Puijalon 2012), and seagrasses (Martin et al. 2015). However, no other studies, to my knowledge, have measured the tensile root strength of emergent wetland macrophytes in the field or conducted experiments on the effects of multiple stressors and their interaction on the tensile strength of an emergent plant species. Therefore, determining the tensile root strength of emergent wetland plants in the Mississippi River Delta (MRD) may guide current and future coastal restoration efforts that would help curtail coastal land loss in Louisiana and other regions.

The objective of this study was to determine the variability in tensile root strength of several coastal wetland plants as a function of soil depth, site, age, and species. I hypothesized that tensile root strength decreased with depth and differed between sites, between live and dead roots, and among species.

MATERIALS AND METHODS

I measured the tensile root strength of five wetland plant species collected from three estuaries in a total of ten samples of vegetation using commercially available equipment that is normally used to measure the tensile strength of cloth fibers.

Study Sites

The samples of vegetation were obtained from three estuaries in southeastern Louisiana (Fig. 2.1, Sites 1A, 1B, 2C, 2D). The Breton Sound Estuary, located approximately 20 km south of New Orleans, LA is comprised of a matrix of fresh, intermediate, brackish, and saline wetlands (CWPRRA 2017a). The dominant vegetation is *Spartina patens* in the lower-salinity marshes and *Spartina alterniflora* in the higher-salinity areas. The Breton Sound Basin is a remnant deltaic lobe of the abandoned St. Bernard Delta of the Mississippi River, which was active 2800 to 1000 years BP (CWPRRA 2017a). The boundaries of the estuary are formed by Bayou La Loutre in the north, Baptiste Collette Bayou and Breton Island in the south, the south bank of the Mississippi River Gulf Outlet (MRGO) in the east, and the west bank of the Mississippi River in the west (CWPRRA 2017a). Anthropogenic impacts, including the dredging of oil and gas canals, the construction of flood protection levees, and the creation of the Mississippi River Gulf Outlet (MRGO) shipping channel, changed the hydrologic and ecological dynamics within the estuary (LPBF 2006). These disturbances resulted in an increase in salinity in the upper estuary that caused a shift in plant communities and conversion of fresh and intermediate marshes to brackish and salt marshes. Dunbar et al. (1992) estimated that 19,035 hectares (ha) of wetlands were converted to open water from1932 to 1990. Also, nutrients introduced by the Caernarvon diversion caused a chronic weakening of soils, which converted to

open water during Hurricane Katrina (Howes et al. 2010). The severe impacts of the hurricane on the basin resulted in the loss of 527 km^2 of wetlands (Kearney et al. 2011).

The second sampling site was located in salt marshes near Port Sulphur, LA in the Barataria Bay Estuary (Fig. 2.1, Site 3). The 633,333 ha Barataria Basin, which is located southwest of New Orleans, LA, is bounded on the north and east by the Mississippi River, on the south by the Gulf of Mexico, and on the west by Bayou Lafourche (CWPRRA 2017a). The upper Barataria Basin was formed approximately 3500 to 4000 years BP by the Lafourche Delta of the Mississippi River (CWPRRA 2017a). The wetlands in the Barataria Bay Estuary consist of bottomland hardwood forests, cypress-tupelo swamps, and a matrix of fresh, intermediate, brackish, and salt marshes (Chabreck 1972). The dominant vegetation in the coastal marshes is *Panicum hemitomon* in freshwater areas, *Spartina patens* in the lower salinity marshes, and *Spartina alterniflora* in the higher salinity areas (Chabreck 1972). The construction of flood protection levees along the Mississippi River and the closure of the Bayou Lafourche distributary reduced the input of freshwater and sediment to the Barataria Basin. The current primary freshwater sources to the basin are precipitation and the three freshwater river diversions at Davis Pond, West Pointe a la Hache, and Naomi (CWPRRA 2017a). The hydrological dynamics of the basin have been disrupted by the dredging of oil and gas canals. Municipal, industrial, and agricultural sources of non-point pollution have degraded the water quality in the basin (LDEQ 2004). Agricultural areas at the head of the estuary are a significant source of nutrients and herbicides that enter the basin via overland runoff. The Barataria Basin lost wetlands at a rate of 2310 ha yr^{-1} between 1974 and 1990 (CWPRRA 2017a).

The third sampling site was a freshwater marsh located 11 km south of Hammond, LA. The marsh is on the northern border of the Joyce Wildlife Management Area (Joyce WMA; Fig.

2.1, Sites 5A, 5B). The City of Hammond, LA began a wastewater discharge of partially treated wastewater effluent (hereafter, effluent) into it in 2006 (Bodker et al. 2015). Before wastewater discharge, the vegetation community of the emergent marsh was co-dominated by *Panicum hemitomon* (Maidencane) and *Sagittaria lancifolia*, which were interspersed among tracts of cypress-tupelo swamp (*Taxodium distichum* and *Nyssa aquatica*, respectively). By 2010, however, 150 ha of the marsh had converted to open water, and the plant community cover had shifted to annual and floating species, two of which were the invasive species *Salvinia molesta* (giant salvinia) and *Ludwigia leptocarpa* (Willow Primrose) (Bodker et al. 2015).

The fourth sampling location, Bayou Sauvage National Wildlife Refuge (Bayou Sauvage NWR; Fig. 2.1, Site 4) in the Lake Pontchartrain basin, is mostly comprised of salt, brackish, intermediate, and fresh marshes dominated by *Spartina alterniflora* and *Spartina patens*. Sixty percent of the Bayou Sauvage NWR is located within the hurricane protection levee system, and water levels within the levee system are managed by the U.S. Army Corps of Engineers (USFWS 2009). Anthropogenic alterations, such as large excavated fill pits, canals, spoil banks and urban runoff, can affect the natural hydrologic regime and nutrient chemistry of the area (CWPRRA 2017b). In addition, overland flow during precipitation events entrains numerous toxicants from the City of New Orleans and the large Resource 1 sanitary landfill adjacent to the refuge, which creates a large source of nonpoint pollution (USFWS 2009). Drought conditions sometimes lower water levels and lead to oxidation of organic soils within the refuge, which facilitates their subsidence (LPBF 2006). Also, the introduction of slightly saline water from Lake Pontchartrain may alter plant community structure (LPBF 2006).

Field Sampling

I extracted two 25–30 cm soil-plant plugs of each of five emergent wetland macrophytes: *Spartina patens, Spartina alterniflora, Schoenoplectus americanus, Sagittaria lancifolia,* and *Panicum hemitomon* (Table 2.1). At the Joyce WMA site, two soil-plant plugs were sampled from the wastewater treatment wetland and from a wetland hydrologically isolated from the Joyce WMA site (Anderson Canal, Table 2.1). The soil-plant plugs were transported to Louisiana State University and transplanted into 5 gallon plastic buckets with holes drilled 1.25 cm above the soil surface and then stored in a holding tank filled with enough water to cover the soil surface.

Table 2.1 Field study sampling sites in southeastern Louisiana wetlands

Map Reference #	Site	Location		Species	Samples
		Longitude (W)	Latitude (N)		
	Breton Sound				
1A	Delacroix	89.762	29.796	*Spartina patens*	2
1B	Delacroix	89.761	29.795	*Schoenoplectus americanus*	2
2C	Yscloskey	89.689	29.839	*Spartina patens*	1
2D	Yscloskey	89.690	29.839	*Spartina patens*	1
	Barataria Basin				
3	Port Sulphur	89.447	29.268	*Spartina alterniflora*	2
	Lake Pontchartrain				
4	Bayou Sauvage NWR	89.873	30.136	*Spartina alterniflora*	2
5A	Anderson Canal	90.425	30.406	*Panicum hemitomon*	2
5B	Treatment Wetland	90.440	30.412	*Panicum hemitomon*	2
5A	Anderson Canal	90.425	30.406	*Sagittaria lancifolia*	2
5B	Treatment Wetland	90.440	30.412	*Sagittaria lancifolia*	2

Tensile Strength Testing

A 10 cm diameter soil core was subsampled from each soil plug within one hour before testing, divided into 10 cm segments, and refrigerated at 4 °C until measurements were performed. Live roots and rhizomes are white and turgid, whereas dead roots are dark and flaccid (Darby and Turner 2008). Five individual root metrics were measured: mass, length, diameter, cross-sectional area, and volume. Root length was measured to the nearest 0.1 mm with a Scale Master© Classic digital planimeter (Calculated Industries, Carson, NV USA). The mean diameter of roots greater than 0.1 mm and less than 2.0 mm was measured to the nearest 0.1 mm with a Starrett digital IP67 micrometer.

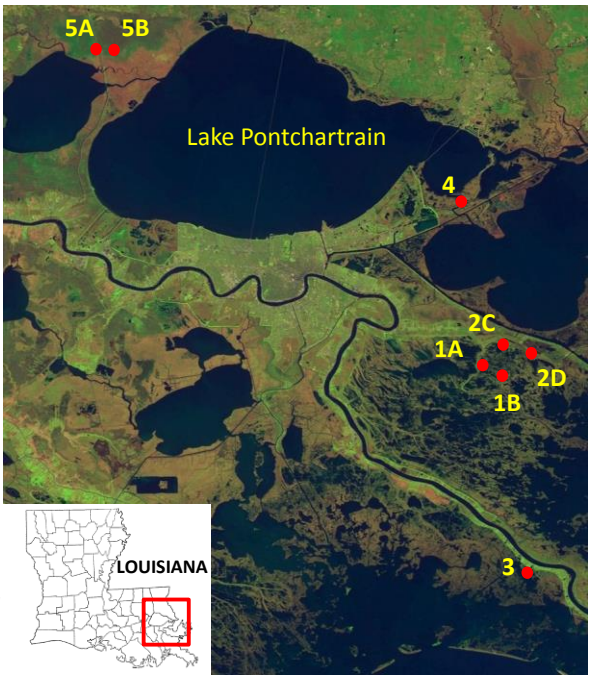


Fig. 2.1 The locations of field sampling sites in the Breton Sound, Barataria, and Lake Pontchartrain basins in southeastern Louisiana (Imagery courtesy of the U.S. Geological Survey)

The diameter and length were the averages of measurements that were taken at both ends and at the middle of each root. The cross-sectional area (mm^2) and volume (mm^3) were calculated from the length and diameter measurements after tensile strength measurements. The

fine root hairs of each test sample were trimmed to 0.16 cm length before measurements. Three roots were destructively sampled to determine the mass (correction factor) of the remaining 0.16 cm projections, which was subtracted from the root mass. Individual root samples were weighed to the nearest 0.1 mg. I used a Mecmesin MultiTest 1–d motorized stand (Mecmesin Limited; Sinfold, West Sussex, United Kingdom) to measure the tensile root strength in Newtons (N). The individual roots were secured to two support clamps that were perpendicular to the base of the test stand (Figure 1). The contact surfaces of the clamps provided 1.25 x 2.50 cm of area and were lined with fine sandpaper to reduce or eliminate slippage. The support clamps were attached to a Mecmesin Basic Force Gage load meter, which was capable of measuring 1000 N of force with a precision of 0.1 N. The test stand was activated and the top support was pulled upward by a vertical hydraulic piston until the root exhibited structural failure. The load that induced failure at that point, or breaking force, was recorded as the tensile strength. The standard engineering practice to determine the strength of materials under tension entails the calculation of either stress (Equation 4) or ultimate tensile strength (Equation 5):

$$\text{Stress, } \sigma = \frac{\text{Force}}{\text{Area(f)}} \qquad (4)$$

where Area equals the cross-sectional area at the point of failure, and

$$\text{(Ultimate) Tensile Strength, } T = \frac{\text{Force}}{\text{Area(i)}} \qquad (5)$$

where Area(i) equals the initial cross-sectional area before the load was applied.

Niklas (1992) cautioned that the tensile strength of materials exhibiting permanent deformation or plastic behavior cannot be calculated with Eq. 2 by using the pre-loading cross-sectional area. This is because the organic materials of the target species are permanently deformed under tension and do not return to their original configuration when the load is removed. Niklas (1992)

stated that the instantaneous cross-sectional area *at the point of failure* must be used to calculate tensile strength. I lacked the equipment to measure root diameter at the point of failure and the software to generate stress-strain curves during testing. Therefore, I used the breaking force (N) at structural failure as a proxy measurement for tensile root strength in a manner similar to the measurement of soil strength using field shear vanes, which measures the torque to estimate soil shear strength. A single soil shear vane measurement cannot estimate or resolve the tensional, compressional, normal, frictional, and tangential forces that are exerted on the soil. The soil shear vane measurement is, therefore, also a proxy metric of soil shear strength. The root data and tensile strength test results were aggregated by site.

Statistical Analyses

I conducted an analysis of variance (ANOVA) using JMP v. 12 software (SAS Cary, NC) to test for differences in the mean tensile strength of roots by depth, site, and whether roots were live or dead. Significant differences between the tensile root strength means were determined using a Tukey-Kramer Honest Significant Difference (HSD) test. The data are reported as the mean ± 1 standard error of the mean ($\mu \pm 1$ SE) unless otherwise noted. The root data were tested for normality using a Shapiro-Wilks test. Homoscedascity was determined with Brown-Forsythe and Levene's tests. Data that did not meet the assumptions of ANOVA were tested with a Welch's ANOVA, and differences between the tensile strength means were determined using a Steel-Dwass nonparametric multiple comparison test. I used a nonparametric Spearman's Rho (ρ) correlation matrix to investigate the relationship between tensile root strength and the root metrics of live and dead roots in each species. I also used a Fisher's z-transformation test to determine whether or not there were significant differences among the Spearman's Rho

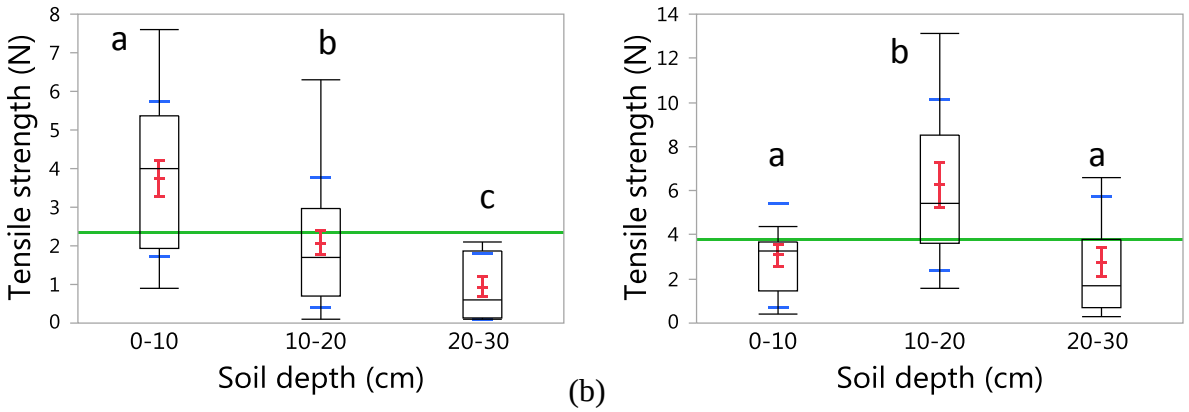
correlation coefficients of the five species. All statistical tests were performed at a significance level of $p < 0.05$.

RESULTS

Spartina patens

The tensile root strength of the dead roots of *Spartina patens* from Yscloskey ranged from a maximum of 7.6 N in the 0–10 cm soil core section to a minimum of 0.1 N in the 20–30 cm core (Fig. 2.2a). The mean tensile strength of dead roots decreased with depth from 3.7 ± 0.39 N (μ ± 1 SE) in the 0–10 cm core, 2.1 ± 0.31 N in the 10–20 cm core, to 0.9 ± 0.48 N in the 20–30 cm core. The results from an analysis of variance (ANOVA) revealed a significant difference in the mean tensile root strength of dead roots between all soil core sections ($F = 10.91$, $p < 0.0001$; Fig. 2.2a). The mean tensile root strength for the site was 2.3 ± 0.22 N, and there was no significant difference in the mean tensile root strength of live roots between soil core sections. The tensile root strength of dead roots from Delacroix ranged from a maximum of 13.1 N in the 10–20 cm soil core section to a minimum of 0.3 N in the 20–30 cm core (Fig. 2.2b). The mean tensile strength of dead roots increased from 3.1 ± 0.64 N in the 0–10 cm core to 6.3 ± 0.78 N in the 10-20 cm core, before decreasing to 2.8 ± 0.67 N in the 20–30 cm core section.

An ANOVA revealed a significant difference in the mean tensile root strength of dead roots between all soil core sections ($F = 6.91$, $p = 0.0021$; Fig. 2.2b).The mean tensile root strength for the site was 3.8 ± 0.40 N. The tensile root strength of live *S. patens* roots at Delacroix ranged from a maximum of 12.1 N in the 0–10 cm soil core section to a minimum of 0.1 N in the 20–30 cm core (Fig. 2.3a).



(a) (b)

Fig. 2.2 Within-plot comparison of the dead tensile root strength at three soil depths for *Spartina patens* at the (a) Yscloskey site, and (b) Delacroix site in the Breton Sound Estuary. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote ± 1 standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between depths

The mean tensile strength of dead roots increased from 3.0 ± 0.72 N in the 0–10 cm core to 5.3 ± 0.94 N in the 10–20 cm core, before decreasing to 0.8 ± 1.58 N in the 20–30 cm core. An analysis of variance (ANOVA) revealed a significant difference in the mean tensile root strength of dead roots between the 10–20 cm soil core section and both the 0–10 and 20–30 cm core sections ($F = 3.47$, $p = 0.039$; Fig. 2.3a). The mean tensile root strength for the site was 3.5 ± 0.54 N. The dead root tensile strength at the 10–20 cm depth for *S. patens* between the Delacroix and Yscloskey sites in Breton Sound Estuary ranged from a maximum of 13.1 N to a minimum of 0.1 N (Fig. 2.3b). The mean tensile root strength at this depth from Delacroix was 6.3 ± 0.68 N, whereas the mean tensile root strength at Yscloskey was considerably weaker at 2.1 ± 0.49 N. The mean tensile strength between the sites for dead roots at the 10–20 cm depth was 3.5 ± 0.39 N.

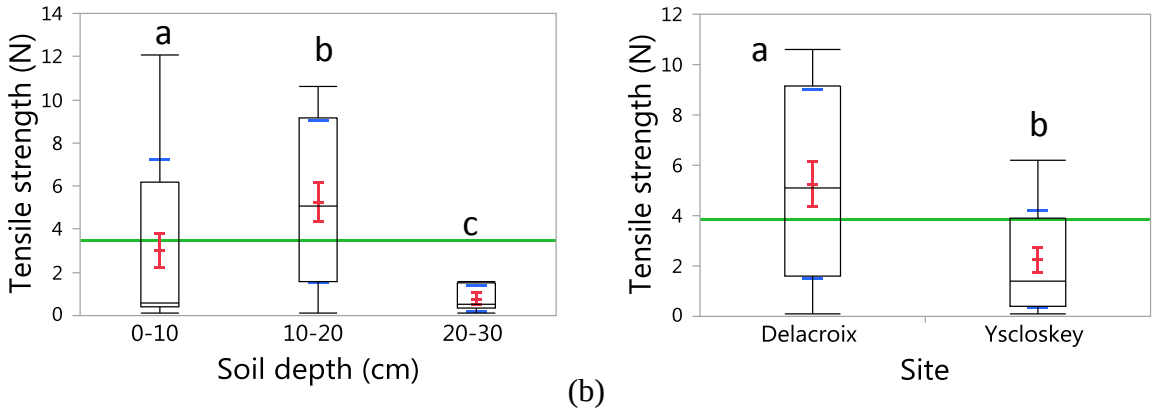


Fig. 2.3 (a) Within-plot comparison of the live tensile root strength at three soil depths for *Spartina patens* from the Delacroix site and (b) site comparison of the live tensile root strength at the 10–20 cm depth for *Spartina patens* between the Delacroix and Yscloskey sites in the Breton Sound Estuary. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote the standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents the standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between depths or sites.

However, the live root tensile strength at the 10–20 cm depth for *S. patens* between the Delacroix and Yscloskey sites in Breton Sound Estuary ranged from a maximum of 10.6 N to a minimum of 0.1 N (Fig. 2.4a). The mean tensile root strength at the 10–20 cm depth was 5.3 ± 0.74 N and 2.3 ± 0.77 N for Delacroix and Yscloskey, respectively. The mean tensile strength between the sites for live roots at the 10–20 cm depth was 3.8 ± 0.55 N. The tensile strength between the Delacroix and Yscloskey sites for dead roots at the 20–30 cm depth ranged from 10.8 to 0.1N (Fig. 2.4b). The mean tensile root strength was 2.8 ± 0.54 N and 0.9 ± 0.69 N for Delacroix and Yscloskey, respectively. The mean tensile strength between the sites for dead roots at the 20–30 cm depth was 2.4 ± 0.42 N.

Schoenoplectus americanus

The mean tensile strength of dead roots at Delacroix decreased with depth from 3.2 ± 0.41 N in the 0–10 cm core to 1.7 ± 0.41 N in the 10-20 cm core ($F = 7.12$, $p = 0.0125$; Fig 2.5a).

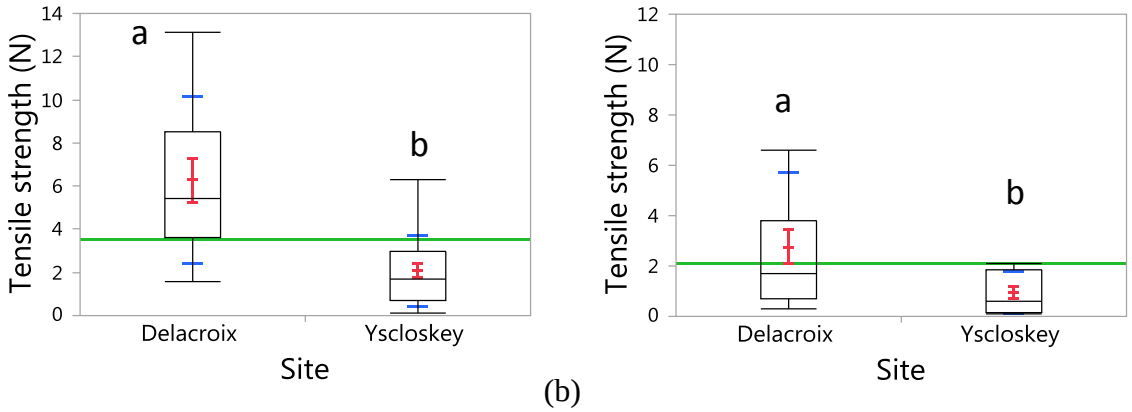


Fig. 2.4 (a) Site comparison of the live root tensile strength at the 10–20 cm depth for *Spartina patens* between Delacroix and Yscloskey sites in the Breton Sound Estuary (b) site comparison of the dead root tensile strength at the 20–30 cm depth for *Spartina patens* between the Delacroix and Yscloskey sites in Breton Sound Estuary. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote the standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents the standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between the sites.

The mean tensile root strength for this plot at the Delacroix site was 2.4 ± 0.29 N. There was a significant difference in tensile root strength between dead and live roots in the 10–20 cm core (F = 6.87, p = 0.0142; Fig 2.5b). The mean tensile strength was 3.2 ± 0.38 N and 1.8 ± 0.37 N for dead and live roots, respectively. The mean tensile root strength for this plot at the Delacroix site was 3.4 ± 0.26 N.

Spartina alterniflora

There was a significant difference in the tensile strength of dead roots in the 10–20 cm core between the Bayou Sauvage NWR and Port Sulphur sites (F = 5.70, p = 0.026; Fig 2.6a). The mean tensile root strength between sites was 2.3 ± 0.38 N. The mean tensile strength of dead roots at Bayou Sauvage NWR decreased with depth from 2.8 ± 0.66 N in the 0–10 cm core to 1.2 ± 0.70 N in the 10–20 cm core (F = 4.32, p = 0.0406; Fig 2.6b). The mean tensile root strength at the site was 2.4 ± 0.48 N.

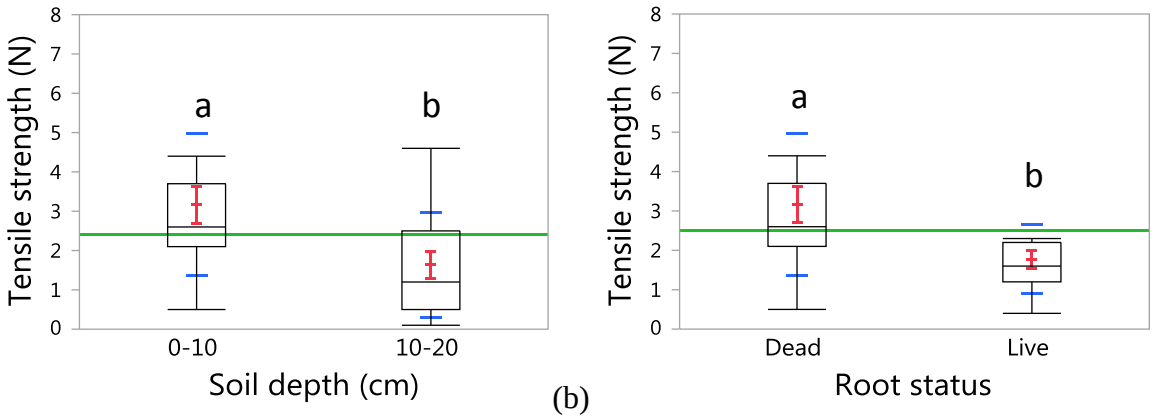


Fig. 2.5 (a) Within-plot comparison of the dead tensile root strength at two depths for *Schoenoplectus americanus* at the Delacroix site in Breton Sound Estuary (b) within-plot comparison of live and dead tensile root strength at the 0–10 cm depth for *Schoenoplectus americanus* at Delacroix in the Breton Sound Estuary. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote the standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents the standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between the groups.

Panicum hemitomon

The *P. hemitomon* root samples at the Joyce WMA did not extend below 10 cm in depth. Plants were, therefore, easily extracted from the marsh soil. There was no evidence that the root mass had been torn away by uplifting forces during sampling. Live roots were concentrated 5–10 cm below the stem, and no dead roots were found in this layer during lab processing and testing. Therefore, because no additional roots were found below the 10 cm soil core section, no comparison of mean tensile root strength could be performed between the treatment wetland and reference wetland samples.

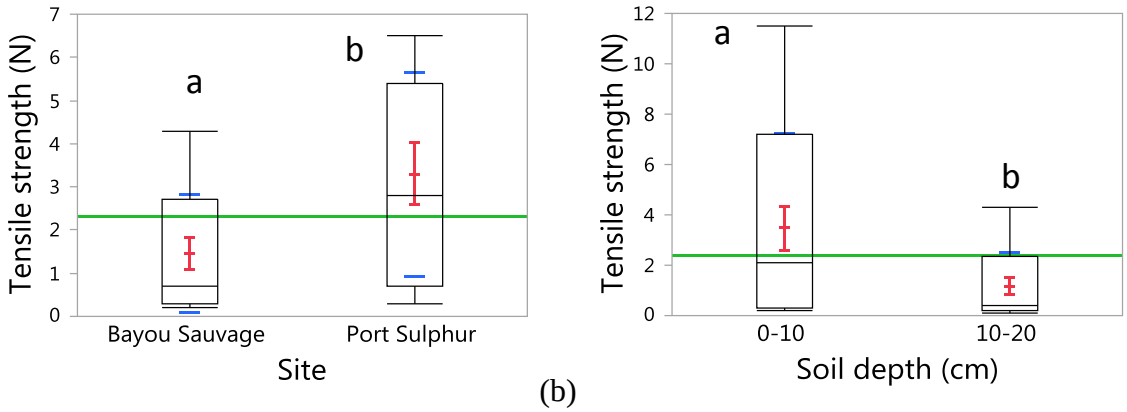


Fig. 2.6 (a) Site comparison of the tensile strength of dead roots at depths 0–10 and 10–20 cm for *Spartina alterniflora* between the Bayou Sauvage NWR site in the Lake Pontchartrain basin and Port Sulphur site in the Barataria Basin (b) within-plot comparison of the dead tensile root strength at two soil depths for *Spartina alterniflora* at Bayou Sauvage NWR in the Lake Pontchartrain basin. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote the standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents the standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between depths.

Sagittaria lancifolia

The *S. lancifolia* samples at the Joyce WMA were found in the same condition as *P. hemitomon* and they were easily extracted from the marsh soil. Also, the belowground biomass did not extend below 10 cm in depth. Roots were concentrated less than 10 cm below the stem, and there was no evidence that the root mass had been severed by uplifting forces during sampling. In addition, no dead roots were found in this 10–cm layer during lab processing and testing, and the tubers were severely atrophied. No additional roots were found below the 10–cm soil core section, therefore, no comparison of mean tensile root strength could be performed between the treatment wetland and reference wetland *S. lancifolia* samples. There were no significant differences between live roots in the 0–10 cm soil core sections from the treatment and reference wetlands. However, the tensile strength of dead roots at the Anderson Canal reference wetland decreased with depth from 3.2 ± 0.64 N in the 0–10 cm core to 1.7 ± 0.64 N in

the 10–20 cm core (F = 7.12, p = 0.053; Fig 2.7). The Type-I error rate was 0.053,which strongly suggests that there is possible biological significance and that tensile root strength decreases with soil depth.

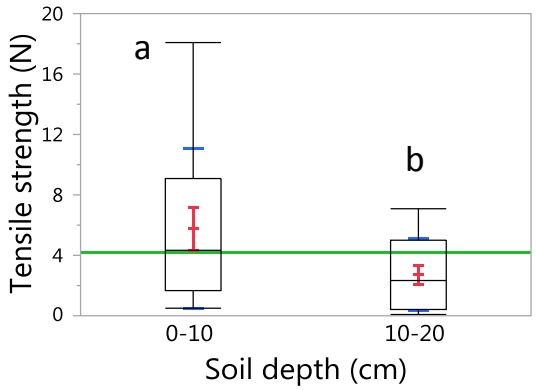


Fig. 2.7 Within-plot comparison of dead root tensile strength at two depths for *Sagittaria lancifolia* at Anderson Canal reference wetland in the Joyce WMA. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote the standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents the standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between depths.

Tensile Strength-Root Metrics Correlation

Table 2.2 is a summary of the correlation between tensile root strength and the diameter, cross-sectional area, and volume root metrics. The results of a multivariate, nonparametric Spearman's Rho correlation matrix revealed that tensile root strength exhibited significant relationships with the root metrics of all five species (Table 2.2, b; $p < 0.0001$). The tensile strength of both live and dead *S. patens* roots had identical and positive correlations with root diameter ($\rho = 0.70$), cross-sectional area ($\rho = 0.69$), and volume ($\rho = 0.70$), respectively (Table 2.2).

Table 2.2. Summary of Spearman's Rho correlation matrix results between the live and dead roots in each of the five species of emergent wetland macrophytes from six sites for tensile root strength vs. root diameter, cross-sectional area, and volume

Species	Root Status	Variable	n	Spearman's ρ	*p*-value
1. Spartina patens	Live	Diameter	114	0.70	<0.0001
1. Spartina patens	Dead	Diameter	111	0.70	<0.0001
1. Spartina patens	Live	Area	114	0.69	<0.0001
1. Spartina patens	Dead	Area	111	0.69	<0.0001
1. Spartina patens	Live	Volume	114	0.70	<0.0001
1. Spartina patens	Dead	Volume	111	0.70	<0.0001
2. Schoenoplectus americanus	Live	Diameter	31	0.66	<0.0001
2. Schoenoplectus americanus	Dead	Diameter	30	0.66	<0.0001
2. Schoenoplectus americanus	Live	Area	31	0.62	<0.0001
2. Schoenoplectus americanus	Dead	Area	30	0.64	<0.0001
2. Schoenoplectus americanus	Live	Volume	31	0.64	<0.0001
2. Schoenoplectus americanus	Dead	Volume	30	0.61	<0.0001
3. Spartina alterniflora	Dead	Diameter	62	0.80	<0.0001
3. Spartina alterniflora	Dead	Area	62	0.80	<0.0001
3. Spartina alterniflora	Dead	Volume	62	0.77	<0.0001
4. Panicum hemitomon	Live	Diameter	75	0.82	<0.0001
5. Sagittaria lancifolia	Live	Diameter	60	0.81	<0.0001
4. Panicum hemitomon	Live	Area	75	0.82	<0.0001
5. Sagittaria lancifolia	Live	Area	60	0.82	<0.0001
4. Panicum hemitomon	Live	Volume	75	0.80	<0.0001
5. Sagittaria lancifolia	Live	Volume	60	0.80	<0.0001
Total Live			**840**		
Total Dead			**609**		
TOTAL			**1449**		

The tensile strength of both live and dead *Schoenoplectus americanus* roots at the Delacroix site had positive correlations with root diameter ($\rho = 0.66$ vs. $\rho = 0.66$), cross-sectional area ($\rho = 0.62$ vs. $\rho = 0.64$), and volume ($\rho = 0.64$ vs. $\rho = 0.61$), respectively (Table 2.2). The tensile strength of dead *S. alterniflora* roots had positive correlations with root diameter ($\rho = 0.80$), cross-sectional area ($\rho = 0.80$), and volume ($\rho = 0.77$), respectively. The tensile strength of live roots of both *Panicum hemitomon and Sagittaria lancifolia* had similar, positive correlations with root diameter ($\rho = 0.82$ vs. $\rho = 0.81$), cross-sectional area ($\rho = 0.82$ vs. $\rho = 0.80$), and volume ($\rho = 0.80$ vs. $\rho = 0.80$), respectively. However, there were no significant differences between the diameter, cross-sectional area, or volume (Spearman's Rho) correlation coefficients of the live and dead roots in the five respective species.

DISCUSSION

The five emergent wetland species that were collected from six sites in southeastern Louisiana exhibited several general characteristics in regard to tensile root strength. These characteristics are discussed next in terms of biomechanical properties, soil depth, root status, site characteristics, and other influences on tensile root strength.

Root Biomechanics

The tensile root strength of both live and dead roots in all five species exhibited a high correlation with increasing root diameter, cross-sectional area, and volume. However, there were no significant differences between the diameter, cross-sectional area, or volume correlation coefficients between live and dead roots among the five species. The lack of any significant differences between the correlation coefficients for live and dead roots among the five species may be related to the similar types of cellular components present within each species, namely cellulose, hemicellulose, lipids, proteins, and polysaccharides. However, this does not imply that

these species are structurally homogeneous. In fact, it is highly unlikely that each species possesses these components in the same proportions. The high correlation with increasing root diameter, cross-sectional area, and volume were contrary to the results of other investigations that had been conducted in terrestrial systems with mineral soils (Wu et al. 1979, Operstein and Frydman 2000, Norris 2005, De Baets et al. 2008) and based on a model conceived by Wu et al. (1979) in which tensile root stress (T_r) decreases with increasing root diameter (D) by a power law equation:

$$T_r = aD^{-b}$$

where (a) is cross-sectional area and (–b) is a species specific constant.

However, the results of the tensile strength tests, in the form of pull-out force measurements, exhibited a linear, positive relationship with root diameter (Osman et al. 2011), whereas tensile stress tests, in which the tensional force is divided by the cross-sectional area, yields a negative relationship between tensile stress and root diameter as noted by the Wu et al. (1979) model. However, biomechanical tests of tensional forces are highly dependent upon *what* is being measured, *how* it is measured, and *when* it is measured. According to Niklas and Spatz (2012), plant materials typically exhibit plastic behavior; that is, they deform under a tensional load and do not recover their original shape. Consequently, to obtain a measurement of *true* tensile stress (Niklas 1992), it is necessary to measure the diameter of the sample (e.g. a root or stem) at the point of failure – not before the test. I found that numerous investigators omitted to note how or when they measured the diameter of their samples to calculate the cross-sectional area. In addition, pertinent details about the capabilities of their test stands' or measuring devices' ability to measure the diameter of samples during testing were also omitted. For instance, Kowalik et al. (2016) did not state *how* they measured the diameter of their root samples or if their test stand

(Instron 5966) was capable of measuring the sample diameter *during* a tensile strength test. Instead, they reported that they measured the *tensile strength* of *Phragmites australis* rhizomes by dividing the *tensile force* (N) by the cross-sectional area of their samples, which was calculated "at the point of rupture" (Kowalik et al. 2016, p. 290) – presumably by measuring the diameter after the root failed, not *when* it failed during the test. I noted during my measurements that root samples leave frayed edges at the point of failure - they do not break 'cleanly'. Therefore post-test measurements may not accurately measure the actual deformation of the sample diameter. The use of a post-test diameter measurement will result in underestimation of tensile stress because a larger diameter, instead of the deformed smaller diameter, will be used in the cross-sectional area calculation. These testing anomalies would seem to confound comparisons with the results of other investigations because of the resulting confusion about what is actually being measured and the technical term used to described those measurements, namely pull-out force, tensile force, tensile strength, tensile stress, or ultimate tensile strength. I was not able to generate stress/strain curves and measure the diameter of root samples during testing while the sample was subjected to deformation under a tensional load. Instead, I used the breaking force (in Newtons) that caused root failure as a proxy measurement for tensile strength, and I denoted it as tensile strength to reduce confusion and to generate results comparable to similar studies.

Depth

The tensile root strength of three out of five species of emergent wetland macrophytes declined with depth at all field sites except at the Joyce WMA. This is a result consistent with use of cotton strip assays conducted under similar field conditions. The cotton strip assay measures cellulose decomposition in soils by assessing the loss of tensile strength with

decomposition (Slocum et al. 2009). The cotton strip is 96% cellulose and used as a proxy for plant biomass (Maltby 1988). Slocum et al. (2009) documented a greater than 70% loss in cotton tensile strength in soil sods from both oligohaline and salt marsh soils over 19 days at a depth of 8 cm. Turner (2011) reported a 16% to 48% loss of tensile strength in canvas strips that were buried for 14 days in fertilized plots located in a Louisiana salt marsh and reduced soil shear strength at depths of 60–100 cm within these same plots. The soil redox potential generally decreases with depth as more anoxic conditions prevail. As a result, the rate of decomposition may be considerably reduced. But, if tensile root strength decreases with depth, then how does this occur when decomposition rates decline? In this study, the tensile strength of dead roots of *S. patens, S. alterniflora, S. americanus,* and *S. lancifolia* decreased with depth from the surface to 30 cm. The decomposition rates at shallow depths may be because of rapid degradation of more labile material from recently senesced roots. In addition, the redox potential may be higher at shallow depths because of oxygen diffusion and/or aerobic/anaerobic interfaces. However, as decomposing roots and other organic matter accrete and increase the depth of peat, older roots are subjected to slower rates of decomposition, but the remaining material may consist of refractory elements with high percentages of cellulose and hemicellulose. Therefore, although deeper roots may decompose at slower rates than at the surface, their declining tensile strength may be due to age. Younger, shallow dead roots may maintain tensile strength despite the relatively rapid loss of labile elements, which leave refractory materials that may possess greater tensile strength. The tensile root strength of live roots may also change due to age as the plant continues to grow or adapts to abiotic conditions or stressors. Consequently, the tensile root strength of emergent wetland macrophytes may have a temporal as well as a spatial component. For example, Bodker et al. (2015) performed three, 30–32 day cotton string decomposition

experiments at the Joyce WMA treatment wetland and recorded a mean tensile strength loss at depths of 0–40 cm of 0.63 and 0.38 percent cotton strip tensile strength loss per day (CTSL) for the treatment and reference wetlands, respectively. Maltby (1988) recorded an increase in cellulose decomposition and CTSL with depth in phosphorus and nitrogen + phosphorus (N+P) treated channels in both the water column and submerged peat in an Everglades marsh over a 14–day period. The ratio of CTSL in submerged peat to the CTSL in the upper 30 cm of the water column varied with distance from the nutrient source and with depth from 1.17 to 5.91 at 65 m from the phosphorus treatment and 1.17 to 2.95 for the N+P treatment at the same distance. The CTSL increase in the submerged peat may have been affected by the presence of an organic matter substrate that was available for microbial respiration (Maltby 1988). Similarly, Maltby (1988) found an increase in CTSL with soil depth in lower Mississippi River floodplain forests at Red River Bay and the Pearl River over a 2–week period. The mean CTSL was roughly 6% from the soil surface to a depth of 20 cm and the cellulose decomposition rate was higher in nutrient-rich, high-pH Red River Bay soils, than in the nutrient-poor, low-pH Pearl River soils (Maltby 1988). The increase in CTSL with depth may have been facilitated by microbial communities that are capable of anaerobic respiration (Maltby 1988).

Dead vs. Live Roots

In general, the dead roots in this study were stronger than the live roots of the same size, and the tensile strength of dead roots decreased with depth for all species at all sites. Vascular plant tissues are comprised of a heterogeneous matrix of soluble compounds of amino acids, sugars, lipids, and proteins as well as structural lignocellulosic components such as lignin, cellulose, and hemicellulose (Niklas 1992, Moran et al. 1989). The ratio of these components varies between species and within the same species as well as within different organs and tissues

of the same species (Niklas 1992, Niklas and Spatz 2012, Moran et al. 1989). In addition, the variation in these components within plant cells and tissues can affect the biomechanical properties of both live and dead roots. Newly emerged roots may be succulent, fragile, and easily broken. However, dead roots, which may have been subjected to decomposition, may be harder, less flexible, and less friable than live roots. Genet et al. (2007) found a significant and positive correlation between the tensile root strength and the percentage of cellulose in roots of the European tree species *Castanea sativa* (Sweet Chestnut). However, Niklas (1992) asserted that because wet cellulose is weaker than dry cellulose, the loss of water from plant cell walls increases the load-bearing capacity of plant tissue. One of the most important biomechanical properties distinguishing dead roots from live roots is turgor pressure. Fully turgid protoplasts exert hydrostatic pressure that places the cell walls in tension and increases the elastic modulus of thin-walled tissue such as parenchyma (Niklas 1992). Live roots may be subjected to constant stresses and strains due to turgor pressure and normal plant development during growth. Conversely, dead roots are usually devoid of turgor pressure and are not subjected to structural modifications due to growth. Under anoxic conditions, live roots employ several adaptations to facilitate gas exchange between the roots and the atmosphere, which alters the root's internal structure. The formation of lacunae, or large air spaces within the cortex of the root, lowers the resistance to gas exchange. However, lacunae create a greater surface area, which allows more tissue to be mobilized by tensile forces. The honeycomb structure of aerenchyma decreases the amount of load-bearing tissue, and tensional forces are transmitted through thinner tissue, which increases the number of structural junctions where failure could occur. Schizogeny and lysigeny may increase the root volume as well as root porosity, which can weaken the structural integrity of internal tissue. Lysigeny involves the splitting of cell walls and cellular collapse, whereas

schizogeny is the enlargement and separation of cells without cellular collapse (Cronk and Fennessy 2001). Individual live roots may be weaker than dead roots, but collectively, both reinforce soil strength. Live roots, fine roots, and root hairs may increase soil-root friction, overall plant-soil cohesion, plant anchorage, and generate greater resistance to shearing forces in the soil.

Site Characteristics

The tensile root strength site means ranged from 2.3 ± 0.38 to 3.8 ± 0.39 N with a 2.9 ± 0.45 N grand mean for all sites. The low variation in mean tensile root strength between sites suggests that there is a common denominator affecting the tensile root strength of these five species at different locations in the Mississippi River Delta (MRD). One common denominator for all sites is the loading of nutrient- and herbicide-laden Mississippi River water and the local sources of nutrient and herbicides that are transported to the estuaries via overland flow and surface conveyances. Another common denominator is the impact of hurricanes and tropical storms. For instance, Howes et al. (2010) noted that 'marshballs' in Breton Sound, which are uprooted masses of marsh vegetation and sediment, had been sheared away from the wetland surface at a depth of 20–30 cm by Hurricane Katrina. In addition, they observed that the majority of the marshballs were formed of eroded swaths of *S. patens*. The soils in the Breton Sound Basin are highly organic hydric soils with low mineral content. The tensile strength of the *S. patens* dead roots from the Yscloskey site was lower than that of the *S. patens* dead roots at the Delacroix site at a depth of 20–30 cm. However, the tensile strength of both live and dead *S. patens* roots from the Delacroix site declined significantly below 20 cm. For each plot, roots from the 10–20 cm core exhibited greater tensile root strength than the roots from depths of 0–10 cm and 20–30 cm, which is inconsistent with a trend of declining tensile root strength with

depth. One possible explanation for these contrasting results is that wetland macrophytes will generate shallow rooting systems as a response to inundation. These live, adventitious roots are often small, new growths, which are usually succulent and fragile. Also, wetland soils are often subjected to numerous stresses that can weaken the top layer of the soil, such as herbivore grazing, wind, and wave action. In addition, the surface layer may have contained an aerobic-anaerobic interface. Exposure to oxygen could have led to oxidation of the soil and increased decomposition rates. The deeper soil layers may escape the effects of the surface stressors, but not the effects of anaerobic conditions that may be present. Roots in these deeper soil layers, at or below 30 cm, may exhibit less shear strength due to the decline in root reinforcement (Howes et al. 2010) or decomposition (Turner 2011). For instance, Graham and Mendelssohn (2014) reported a considerable decrease in the soil shear strength at the 15–25 cm depth in fertilized plots in an oligohaline marsh located in the northern Lake Pontchartrain basin. However, in this study, the tensile strength of dead roots from Yscloskey at the 10–20 and 20–30 cm depths were considerably weaker than the Delacroix samples. The very distinct aroma of hydrogen sulfide (H_2S) was detected at both the Yscloskey and Delacroix sites during sampling, which may have been an indication of decreasing redox potential with depth. The difference in mean tensile strength between the sites may have existed because of differential rates of decomposition and the age of the roots at lower depths.

The mean tensile root strength of dead *S. alterniflora* roots at Bayou Sauvage NWR (1.3 N, Fig. 2.4b) was less than half of that of *S. alterniflora* dead roots at Port Sulphur (3.2 N, Fig. 2.4b). However, the Bayou Sauvage NWR is more protected from the biomechanical stresses due to wind and wave action, whereas the Port Sulphur site is exposed to considerable fetch and the open waters of Breton Sound. Nevertheless, the tensile root strength of *S. alterniflora* at Port

Sulphur was twice that of *S. alterniflora* at Bayou Sauvage NWR. As previously mentioned, the Bayou Sauvage NWR is located entirely within the City of New Orleans. The USFWS (2009) reported that the refuge can be subjected to urban nonpoint pollution during run-off events in which overland flow from urban areas can entrain these potential hazards. Consequently, the tensile root strength of the belowground biomass of *S. alterniflora* at Bayou Sauvage NWR may have been diminished in a manner similar to *P. hemitomon* that was exposed to wastewater effluent at the Joyce WMA treatment wetland.

The 53–ha portion of the City of Hammond treatment wetland that is adjacent to the Joyce WMA was subjected to an average discharge of 14,498 m^3 d^{-1} of wastewater effluent from 2006 to 2008, and the average total nitrogen (TN) and total phosphorous (TP) concentrations in the effluent were 16.90 and 3.23 mg L^{-1}, respectively (Bodker et al. 2015). Figure 2.8 illustrates the striking differences between a *P. hemitomon* treatment wetland sample (Fig. 2.8, left) and a reference marsh sample (Fig. 2.8, right). This exposure to effluent may have substantial biomechanical consequences for the plant species at this site. First, the belowground biomass of the treatment marsh sample was substantially diminished because there were fewer roots, fewer live roots, smaller roots, and shorter roots than at the reference site. As a result, plants in this condition will have less soil-root friction, lower plant-soil cohesion, and lower soil shear strength. Samples in this or similar condition were easily pulled out of the soil by hand and with little effort, whereas samples from the reference site had to be laboriously excavated. Second, the aboveground biomass of the treatment marsh sample was noticeably more robust than the reference site sample, with larger, greener, and more numerous stems. The robust aboveground biomass could increase the tensile loading on the diminished belowground biomass due to the greater mass and surface area of the stem and leaves that may be mobilized by wind or wave

action, which could increase pull-out and tensional forces on the root mass and lead to dislodgement of the vegetation.

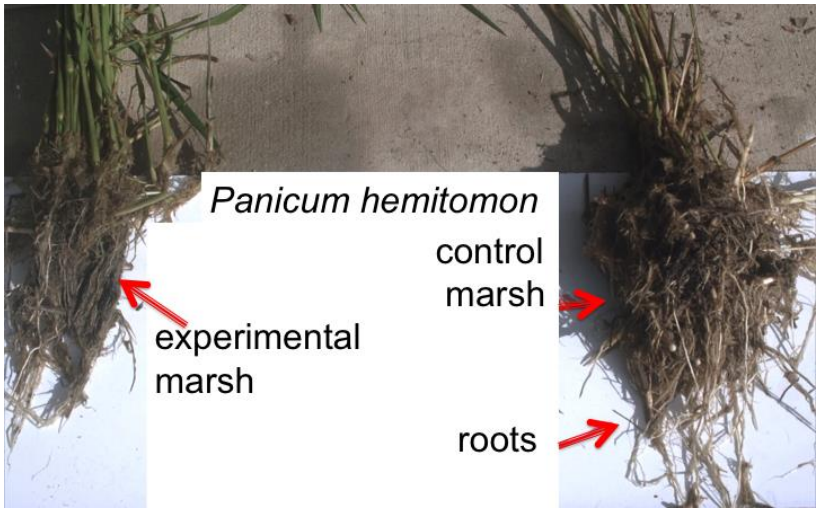


Fig 2.8 A comparison of two *P. hemitomon* samples from Joyce WMA. The sample on the left was obtained from the treatment wetland and the right sample was extracted from a reference site that was isolated from wastewater effluent (Photo courtesy of James E. Bodker)

Other Influences

Site-specific factors such as temperature, pH, redox, flood duration, nutrients, xenobiotics, and microbial communities can influence tensile root strength of individual species in numerous ways. The rate of biochemical reactions of plants and microbes can double for every 10°C increase in temperature (Reddy and Delaune 2008). As a result, the use of carbon as an electron donor may reduce the structural properties of a plant because of the loss of biomass and negatively affect tensile root strength. In addition, increased water temperature can increase biological oxygen demand in the water column or soil pore water and thereby reduce the amount of oxygen available to diffuse into the rhizosphere. Radial oxygen loss from the roots can inhibit the formation of reduced compounds that are toxic to plants. Seasonal ambient temperatures may also affect tensile root strength. For example, Kowalik et al. (2016) examined the tensile properties of the rhizomes of *Phragmites australis* and *Glyceria maxima* in the eutrophic Lake

Urszulewskie in Poland. The rhizome tensile strengths (N) of both species exhibited positive correlations and linear relationships with root cross-sectional area. During the summer, the tensile strength of the rhizomes varied from 25 to 70 N and 95 to 295 N for *P. australis* and *G. maxima*, respectively (Kowalik et al. 2016). During the winter, however, the rhizome tensile strength varied from 42 to 70 N and 110 to 254 N for *P. australis* and *G. maxima*, respectively (Kowalik et al. 2016). They surmised that low temperatures, which could have caused shrinkage of internal rhizomatous tissues, may have contributed to lower tensile strength. The amount of soil moisture can influence tensile root strength. Ennos (1990) found that the roots of leek seedlings (*Allium porrum*) withstood more tensile force (0.35 N) in dry soils and failed at shorter lengths than roots in wet soil (0.18 to 0.22 N). Similarly, Yang et al. (2016) found that the tensile root strength of four tree species in Wudaohe Linchang, China increased with moderate moisture content and increasing diameter from a range of 0.1 to 1.2 N. One of the greatest effects of flooding on tensile root strength may be the formation of aerenchyma tissue, which facilitates gas exchange. Although adaptation elicits survival in anoxic conditions, weakened structural integrity could lead to uprooting or damage from biotic and abiotic forces that would preclude reproduction and propagation of the species. Lamberti-Raverot and Puijalon (2012) asserted that wetland plants sacrifice structural integrity for the ability to persist in anaerobic conditions because the formation of aerenchyma reduces resistance to tensile and compressive forces. Therefore, an ecological trade-off exists as plants risk fitness in order to survive stressful conditions such as anoxia and reducing conditions in the soil.

Redox potential determines the sequential reduction of alternate electron acceptors that may be used for anabolic or catabolic metabolic processes. These oxidation-reduction reactions require an electron donor that is usually a carbon substrate derived from plant material. The loss

of carbon can reduce the amount of internal material of a root and weaken its structural integrity. For example, the redox potential plays a role in the formation of the plant hormone ethylene, which requires carbon for its synthesis. Ethylene, in turn, plays an important role in the formation of aerenchyma tissue. The increase in ethylene concentration under anaerobic conditions stimulates structural degradation in cell walls, which is a process that can directly affect tensile root strength (Cronk and Fennessy 2001).

The pH can affect tensile root strength through its influence on the availability of some nutrients that can affect processes such as respiration and denitrification. The release of nutrients, together with the use of carbon as an electron donor, can change the internal structure of the roots. Phosphorus is in its most plant-available form when the pH is between 6 and 7, and under alkaline conditions, P can form precipitates with calcium; whereas under acidic conditions, P forms metal complexes with Fe and Al compounds in the soil (Reddy and Delaune 2008). The combination of excess phosphorus and nitrate has been shown to increase the rate of decomposition of the belowground biomass of wetland plants (Darby and Turner 2008). Faster decomposition rates could result in structural changes in the root and alter tensile root strength. Changes in nutrient availability may also affect the composition of microbial communities, which mediate nutrient concentrations through dissimilatory and assimilatory reduction of nitrogen. A shift in microbial communities, such as one populated by the genera *Nitrosomonas* and *Nitrobacter*, may determine the utilization of nitrate as an alternate electron acceptor, and hence, the progression of oxidation-reduction reactions. Also, some xenobiotics, such as the herbicide atrazine, may be a nutrient source for plants. During the mineralization of the atrazine molecule, the cyanuric acid ring may be degraded to urea, and urea, via ammonification and/or nitrification, can become a source of nitrogen for wetland plants. Consequently, atrazine may be

an additional source of nitrogen, which could exacerbate eutrophic conditions in coastal estuaries and marshes.

CONCLUSIONS

The tensile root strength of three ubiquitous wetland plants declined with depth in brackish and salt marshes in the MRD. The tensile root strength of S. *alterniflora, S. patens,* and *Schoenoplectus americanus* became weaker at depths of 20–30 cm. In general, dead roots were stronger than live roots of the same diameter. The decomposition of labile root components and the persistence of more refractory materials may contribute to the difference in tensile strength between live and dead roots. In addition, there were noticeable differences in tensile root strength between the sites. The narrow range of the overall (site) mean tensile strength at all sites may indicate that roots of these important and dominant wetland plant species are being degraded by a common stressor. These sites are receiving basins for either direct or diverted Mississippi River flow, which has been widely reported to contain high nutrient loads and numerous xenobiotics such as pesticides and herbicides. The degraded condition of the *P. hemitomon* (Fig. 2.7) root samples at the Joyce WMA provide considerable evidence that poor water quality may be a factor in the declining tensile root strength of emergent wetland plants in the MRD. Consequently, management efforts should be reallocated to address water quality issues in riverine watersheds and the coastal zone. In addition, the health of inland and upstream wetlands may play an important role in mitigating poor water quality in the coastal wetlands. Wetland restoration efforts in the coastal zone and at inland areas of coastal watersheds may increase interception, sequestration, and/or transformation of xenobiotics that could adversely affect the structural stability of coastal wetlands. Field and/or greenhouse experiments would be highly useful in identifying the agent or agents that may be responsible for the loss of tensile root

strength of emergent macrophytes. Coastal wetlands that are degraded by eutrophic or contaminated inflows can reduce the quality of primary and nursery habitat for species at multiple trophic levels, including commercially valuable species. Emergent wetland vegetation and phytoplankton are two important primary producers that support wetland and estuarine ecosystems. Researchers have found that atrazine can reduce the growth of some species of wetland plants (Bouldin et al. 2006) and negatively affect phytoplankton communities (Starr et al. 2017). As a result, eutrophic water quality and xenobiotics may cause a cascading series of negative effects that could induce further changes at higher tropic levels.

REFERENCES

Baustian J, Mendelssohn IA, Lin Q, Rapp J (2010) In-situ burning restores the ecological function and structure of an oil-Impacted coastal marsh. Environmental Management 46:781–789 DOI 10.1007/s00267-010-9549-4

Bodker JE, Turner RE, Tweel A, Schulz C, Swarzenski C (2015) Nutrient-enhanced decomposition of plant biomass in a freshwater wetland. Aquatic Botany 127:44-52

Bouldin JL, Farris JL, Moore MT, Smith Jr. S, Cooper CM (2006) Hydroponic uptake of atrazine and lambda-cyhalothrin in *Juncus effusus* and Ludwigia peploides. Chemosphere 65:1049-1057

Chabreck RH (1972) Vegetation, water and soil characteristics of the Louisiana coastal region. Bulletin No. 664. Louisiana State University Press, Baton Rouge 72 pp.

Coastal Wetlands Planning, Protection and Restoration Act (CWPPRA) (2017a) The Breton Sound Basin: Summary of Basin Plan. https://lacoast.gov/new/About/Basin_data/bs/Default.aspx. Accessed March 15, 2017

Coastal Wetlands Planning, Protection and Restoration Act (CWPPRA) (2017b) The Barataria Basin: Summary of Basin Plan. https://lacoast.gov/new/About/Basin_data/ba/Default.aspx. Accessed March 24, 2017

Comino E, Druetta A (2010) The effect of *Poaceae* roots on the shear strength of soils in the Italian alpine environment. Soil & Tillage Research 106:194-201

Comino E, Marengo P, Rolli V (2010) Root reinforcement effect of different grass species: A comparison between experimental and models results. Soil & Tillage Research 110:60–68

Cronk JK, Fennessy MS (2001) Wetland Plants: Biology and Ecology. CRC Press, New York, NY 462 pp.

Crouzy B, Edmaier K, Perona P (2014) Biomechanics of plant anchorage at an early development stage. Journal of Theoretical Biology 363:22-29

Darby FA, Turner RE (2008) Below- and aboveground *Spartina alterniflora* production in a Louisiana salt marsh. Estuaries and Coasts 31:223–231

De Baets S, Poesen J, Reubens B, Wemans K, De Baerdemaeker, Muys B (2008) Root tensile strength and root distribution of typical Mediterranean plant species and their contribution to soil shear strength. Plant and Soil 305:207-226

Dunbar JB, Britsch LD, Kemp EB (1992) Land loss rates, report 3, Louisiana coastal plain. U.S. Army Corps of Engineers Technical Report GL-90-2, 27 pp.

Easson DL, Pickles SJ, White EM (1995) A study of the tensile force required to pull wheat roots from soil. Annals of Applied Biology 127:363-375

Ennos AR (1989) The mechanics of anchorage in seedlings of sunflower, *Helianthus annuus* L.. New Phytologist 113:185-192

Ennos AR (1990) The anchorage of leek seedlings: The effect of root length and soil strength. Annals of Botany 65:409-416

Fan CC, Su CF (2008) Role of roots in the shear strength of root-reinforced soils with high moisture content. Ecological Engineering 33:157-166

Genet M, Stokes A, Salin F, Mickovski SB, Fourcaud T, Dumail JF, van Beek R (2007) The influence of cellulose content on tensile strength in tree roots. Plant and Soil 278:1–9

Graham SA, Mendelssohn IA (2014). Multiple levels of nitrogen applied to an oligohaline marsh identify a plant community response sequence to eutrophication. Marine Ecology Progress Series 417:73–82

Howes NC, FitzGerald DM, Hughes ZJ, Georgiou IY, Kulp MA, Miner MD, Smith JM, Barras, JA (2010) Hurricane induced failure of low salinity wetlands. Proceedings of the National Academy of Sciences (USA) 107:14014-14019 www.pnas.org/cgi/doi/10.1073/pnas.0914582107

Jain RK (2013) Study on shear strength of soil in relation to plant roots as a combined matrix. International Journal of Applied Sciences & Engineering 1(2):103-130

Kearney MS, Riter CA, R.E. Turner RE (2011) Freshwater diversions for marsh restoration in Louisiana: Twenty-six years of changing vegetative cover and marsh area. Geophysical Research Letters 38, L16405, doi:10.1029/2011GL047847

Kowalik W, Pachuta K, Jeznach J (2016) Selected biometric and mechanical properties of the common reed *Phragmites australis* and the reed sweet grass *Glyceria maxima* rhizomes. Annals of Warsaw University of Life Sciences Land Reclamation 48(4):289–297

Lake Pontchartrain Basin Foundation (LPBF) (2006) Comprehensive Habitat Management Plan for the Lake Pontchartrain Basin, 142 pp.

Lamberti-Raverot B, Puijalon S (2012) Nutrient enrichment affects the mechanical resistance of aquatic plants. Journal of Experimental Botany 63(17):6115-6123

Louisiana Department of Water Quality (LDWQ) (2004) Louisiana Water Quality Inventory: Integrated Report. Water Quality Assessment Division, Standards Assessment and Nonpoint Source Section. Baton Rouge, LA. 110 pp.

Maltby E. (1988) Use of cotton strip assay in wetland and upland environments – an international perspective. *In*: Harrison, A.F., Latter, P.M., Walton, D.W.H. (Eds.), Cotton Strip Assay: An Index of Decomposition in Soils. Institute of Terrestrial Ecology, Grange-Over-Sands, UK, pp. 140–154

Martin CW, Hollis LO, Turner RE (2015) Effects of oil-contaminated sediments on submerged vegetation: An experimental assessment of *Ruppia maritima*. PLoS ONE 10(10):e0138797. doi:10.1371/journal.pone.0138797

Mickovski SB, Bengough AG, Bransby MF, Davies MCR, Hallett PD, Sonnenberg R (2007) Material stiffness, branching pattern and soil matric potential affect the pullout resistance of model root systems. European Journal of Soil Science 58:1471–1481

Moran MA, Benner R, Hodson RE (1989) Kinetics of microbial degradation of vascular plant material in two wetland ecosystems. Oecologia 79:158-167

Muntohar AS (2012) Effect of the roots densities on the shear strength of root-reinforced soil. Seminar Nasional VIII - 2012 Teknik Sipil ITS Surabaya Pembangunan Berkelanjutan Transportasi dan Infrastruktur

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago 608 pp.

Niklas KJ, Spatz HC (2012) Plant Physics. University of Chicago Press, Chicago 426 pp.

Norris JE (2005) Root reinforcement by hawthorn and oak roots on a highway cut-slope in Southern England. Plant Soil 278:43–53

Nyambane OS, Mwea SK (2011) Root tensile strength of 3 typical plant species and their contribution to soil shear strength; a case study: Sasumua Backslope, Nyandarua District, Kenya. Journal of Civil Engineering Research and Practice 8(1):57–73

Operstein V, Frydman S (2000) The influence of vegetation on soil strength. Proceedings of the Institution of Civil Engineers - Ground Improvement 4:81–89

Osman N, Abdullah MN, Abdullah CH (2011) Pull-out and tensile strength properties of two selected tropical trees. Sains Malaysiana 40(6):577–585

Pollen N (2007) Temporal and spatial variability in root reinforcement of streambanks: Accounting for soil shear strength and moisture. Catena 69:197–205

Puijalon S, Bornette G, Lena JP (2007) Interactive effects of nutrient and mechanical stresses on plant morphology. Annals of Botany 100:1297–1305

Puijalon S, Piola F, Bornette G (2008) Abiotic stresses increase plant regeneration ability. Evolutionary Ecology 22:493–506

Reddy KR, Delaune RD (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, New York 774 pp.

Slocum, MG, Roberts J, Mendelssohn IA (2009) Artist canvas as a new standard for the cotton-strip assay. Journal of Plant Nutrition and Soil Science 172:71–74

Starr AV, Bargu S, Maiti K, DeLaune RD (2017) The effect of atrazine on Louisiana Gulf Coast estuarine phytoplankton. Environmental Contamination and Toxicology 72:178–188

Turner RE (2011) Beneath the salt marsh canopy: Loss of soil strength with increasing nutrient loads. Estuaries and Coasts 33:1084-1093. doi: 10.1007/s12237-010-9341-y

United States Fish and Wildlife Service (USFWS) (2009) Bayou Sauvage National Wildlife Refuge Comprehensive Conservation Plan, 166 pp.

Wigand C, Brennan P, Stolt M, Holt M, Ryba S (2009) Soil respiration rates in coastal marshes subject to increasing watershed nitrogen loads in southern New England, USA. Wetlands 29(3):952–963

Wu TH, McKinnell WP III, Swanston DN (1979) Strength of tree roots and landslides on Prince of Wales Island, Alaska. Canadian Geotechnical Journal 16:19–33

Yang Y, Chen L, Li N, Zhang Q (2016) Effect of root moisture content and diameter on root tensile properties. PLoS ONE 11(3): e0151791 doi:10.1371/journal.pone.0151791

CHAPTER 3
THE TENSILE ROOT STRENGTH OF *SPARTINA PATENS* VARIES WITH SOIL TEXTURE AND ATRAZINE CONCENTRATION

INTRODUCTION

Atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine), a member of the s-triazine herbicide family, is widely used in agriculture, is long-lived and is mobile in surface and subsurface waters (Clay et al. 1988). Atrazine is the most frequently detected contaminant in streams in the midwestern United States and was detected at all nine water quality stations in the lower Mississippi River watershed after major flooding events in 1993 and 2011 (Goolsby et al. 1993, Welch et al. 2014). Atrazine adsorption increases under acidic soil conditions and decreases under alkaline soil conditions (Harris and Warren 1964, McGlamery and Slife 1966, Laird and Koskinen 2008). Many studies have demonstrated that humic acids, fulvic acids and organic matter can exert considerable influence on atrazine adsorption and desorption (Frissel 1961, Harris and Warren 1964, McGlamery and Slife 1966, Weber et al. 1969, Hayes 1970, Stevenson 1972, Senesi and Testini 1982, Borggaard and Streibig 1988, Laird et al. 1994). The typically higher organic content of wetland soils increases the affinity of organic matter for the herbicide. Meakins et al. (1995), for example, investigated the mobility, partitioning, and degradation of atrazine in soil and water samples from salt marshes in the watershed of the River Blackwater in Essex, UK and found negligible adsorption of atrazine onto suspended solids at total suspended solids (TSS) concentrations as high as 4 g L^{-1}.

Atrazine degradation in aerobic mineral soils typically increases with high soil temperatures and moisture, whereas degradation decreases with depth, lower pH, lower temperature, and lower soil moisture. McCormick and Hiltbold (1966), for example, reported that the rate of atrazine decomposition doubled with each 10 °C increase in temperature from 10°

to 30 °C. Meakins et al. (1995) found that the atrazine metabolites DEA and DIA were detectable in both the vegetated marsh and mudflat soil cores 8 days after atrazine treatments, and that there was a greater degree of vertical migration of atrazine and its metabolites in mudflat soil cores than in the vegetated marsh soil cores. The movement of the metabolites can be quite complex. Mersie and Seybold (1996), for example, characterized the adsorption and desorption of atrazine and its metabolites DEA, DIA, and HA on Levy soil, which is a silt loam tidal wetland soil from the James River watershed in Virginia. The adsorption coefficients (K_f) indicated that HA was more strongly adsorbed (109 µmol $L^{-1}kg^{-1}$) than atrazine (38.6 µmol $L^{-1}kg^{-1}$), DIA (26.3 µmol $L^{-1}kg^{-1}$), or DEA (22.1 µmol $L^{-1}kg^{-1}$) and that the amount desorbed was greater for DEA (29%) than for atrazine (24%), DIA (23%), and HA (16%). The larger K_f value for HA and the lower percentage of HA desorbed indicates that it is less likely to migrate within the Levy soil compared to atrazine and its other metabolites. Larsen et al. (2001) reported that less than 2% of atrazine was mineralized in sandy, sandy peat, and peat slurries that were created with groundwater under aerobic, denitrifying, sulfate-reducing, and methanogenic redox conditions. They concluded that the aromatic rings of atrazine were not severed under anaerobic conditions, which prevented subsequent atrazine degradation and complete mineralization.

The widespread dispersal of atrazine, varied degradation rates, and its metabolites can pose risks to plants, amphibians, fish, and aquatic invertebrates (USEPA 2016). Lytle and Lytle (1998) exposed the wetland macrophytes *Spartina alterniflora* and *Juncus roemerianus* to three concentrations of atrazine (0.03, 0.25, and 3 mg L^{-1}) during a 5-week greenhouse experiment. They found that the mean shoot growth of *J. roemerianus* was inhibited by the two highest atrazine concentrations, whereas *S. alterniflora* growth was curtailed only during the first week of the experiment. As a result of these experiments, they concluded that *S. alterniflora* was

tolerant of high concentrations of atrazine, which would be lethal to *J. roemerianus.* Cejudo-Espinosa et al. (2009) measured the accumulation and dispersal of three concentrations atrazine (4, 17, and 30 mg L^{-1}) in a greenhouse experiment with *Sagittaria lancifolia, Typha domingensis,* and *Echinochloa pyramidalis* over three exposure periods (1, 3, and 5 days) under a semi-flooded hydrologic regime. Atrazine was assimilated by all three species in less than 10 minutes, and there may have been species-specific factors that determined the magnitude and behavior of herbicide uptake. Atrazine may also affect other types of hydrophyte communities (Lee et al. 1995).

Plant roots interface directly with the soil containing a mixture of atrazine and degradation products, which suggests that there are potential negative effects on plant root growth and strength. Two studies have provided evidence that atrazine can weaken the tensile root strength of emergent macrophytes. First, Turner and Dickens (1987) applied 3 rates of atrazine treatment (0.6, 1.1, 2.2 kg ha^{-1}) to 1.5 x 4.5 m plots of *Eremochloa ophiuroides* (Centepedegrass) that were cultivated in sandy loam and silt loam soils under acidic conditions (pH 5.3–5.8) during a 3-year experiment. Nutrients, in the form of ground limestone, potassium chloride (KCl), and ammonium nitrate (NH_4NO_3), were applied on 15 May ± 3 days, 8 July ± 1 day, and 9 August ± 1 day (presumably during each year) at rates of 2200, 40, and 50 kg ha^{-1}, respectively, on sites that had "medium levels of available potassium and high levels of available phosphorus, magnesium, and calcium" (Turner and Dickens 1987). They found that the tensile strength of *E. ophiuroides* sod blocks decreased linearly with increasing rates of atrazine application. In addition, they reported that visual injury to the grass was greater with a 2–week application interval than with a 4–week interval. A second study by Turner et al. (1990) involved measuring the tensile strength of *E. ophiuroides* sod blocks grown in a silt loam soil under acidic

conditions (pH 5.2–5.6) in 1.8 x 3.7 m plots that received a monthly rate of 0.24 kg N per 100 m^2 from May through August. Atrazine was applied at rates of 2.2 and 3.4 kg ha^{-1}, and 2000-cm^2 blocks were harvested from each plot 2, 4, and 8 weeks after atrazine treatment. They found no significant difference in the tensile strength of sod blocks extracted from the 2- and 4-week plots at both treatment levels and Control for either year; however, there was a statistically significant difference in tensile strength between the 1987 eight-week harvest, 2.2 kg ha^{-1}experimental treatments and the Control (Table 2, Turner et al. 1990).

The results of these two non-wetland plant studies indicate that atrazine could potentially weaken the strength of the belowground biomass emergent wetland macrophytes, but I am aware no previous studies of this possibility. The belowground biomass structure holds the soil together against the erosive forces of wind, waves, and the uplifting buoyancy from flooding, and it contributes to the vertical accumulation of marsh soil (Bodker et al. 2015, Turner 2011). When wetland macrophytes are 'loaded' with these surface forces, the aboveground biomass may act as a lever and transmit torsional, compressional, and tensile forces to the belowground biomass (Niklas 1992, Niklas and Spatz 2012). Tensile strength is the resistance of material in tension to an external load (Niklas 1992, Niklas and Spatz 2012). Quantifying the tensile root strength of emergent wetland plants in different soil textures is an important consideration for habitat conservation and management because tensile strength may be an effective metric to understand coastal wetland health, and resilience to potential perturbations. *Spartina patens* (Ait.) Muhl. is an emergent macrophyte that occupies a large proportion of coastal wetland plant communities in the eastern US, and 96% of Louisiana's coastal wetlands (Chabreck 1972); it is exposed to atrazine after agricultural harvesting operations in the Midwest and the Mississippi River Delta. This study examined the effects of atrazine and different soil textures on the root strength of the

wetland macrophyte *Spartina patens*. It reports on the results from two experiments examining the effects of interactions between atrazine and different soil textures using the tensile root strength of *Spartina patens* as the main metric of response. It tested the hypotheses that both atrazine and soil texture produce synergistic effects that reduce tensile root strength.

MATERIALS AND METHODS

Greenhouse Experiments

Two atrazine exposure experiments were conducted in greenhouses under natural light conditions. *Spartina patens* plugs from Tampa Bay estuary were purchased from Green Seasons Nursery (Tampa, Fl.). Each plug consisted of 7–12 stems growing from a 3.0 x 3.0 x 6.6 cm root mass. These plants did not have a pre-experiment exposure to atrazine. The samples arrived in June 2015 and were transplanted to 3.78-liter glass jars containing various combinations of organic sphagnum peat, clay/silt, and sand according to requirements of each experiment. Sand, silt, and clay components were obtained by Louisiana State University (LSU) greenhouse staff from soil in the Sterlington soil series (coarse-silty, mixed thermic Typic Hapludalfs) located in the Mississippi River floodplain in West Baton Rouge Parish, LA. The soil texture of clay/silt components was estimated by a texture-by-feel field technique (Brady and Weil 2002) and determined to be sandy clay loam. After transplantation, deionized water was added to the experimental treatments until the soil was saturated at the surface. The transplants acclimated for 6–8 weeks to adjust to greenhouse conditions. Glass pots were randomly assigned positions and rotated on a reverse-orientation basis after every treatment period (e.g., south to north, west to east) to reduce the variation in environmental conditions.

In the first experiment, 3.78-L glass jars were filled with 3.0 L of a mixture (by volume) of 65% sphagnum peat (Premier Sphagnum Peat Moss; 100% Canadian peat moss, no added

fertilizer or nutrients), 30% clay/silt, and 5% sand. A 25 ppm atrazine stock solution was formed by placing Pestanal® Sigma-ALDRICH atrazine in deionized water (Starr et al. 2017). Because atrazine has a moderate solubility in water (30 ppm at 20 °C), the solution was placed on a hot plate with a magnetic stirrer, heated at 23 °C, and mixed with magnetic stirring rods for a 24 hour period before the experiment to ensure the atrazine was fully dissolved (Starr et al. 2017). The volume of atrazine required for each experiment treatment (V_2) was calculated by the equation $C_1V_1 = C_2V_2$, where C_1 and C_2 are the initial and final concentrations, respectively; and V_1 and V_2 are the initial and final volumes, respectively. The experimental treatments consisted of four levels of treatments of atrazine (Control: 0 micrograms per liter (µg L^{-1}), Low: 0.5 µg L^{-1}, Medium: 1.5 µg L^{-1}, and High: 3.0 (µg L^{-1}) with four replicates each for a total of 16 experimental treatments. Atrazine treatments were added weekly in a 1 L deionized water solution. Water levels were maintained 1.75 cm above the soil surface to ensure saturated soil conditions. Soil temperature, pH, and redox potential were measured weekly, before the addition of atrazine treatments. A soil probe thermometer was inserted into each unit and the temperature recorded to the nearest 0.1° C. A 175 mL sample of soil pore water was extracted with an improvised pore water sampler and dispensed into a 250 mL amber glass bottle. The sampler consisted of a Lisle vacuum pump (Lisle Corporation, Clarinda, IA) with an intake line composed of Teflon tubing that was secured to a 15 cm metal probe. The pH was measured by a Hach HQ 40d multi-parameter meter (Hach Industries Loveland, CO). Redox potential was measured with 45 cm-long standard platinum probes after Reddy and Delaune (2008) and a Corning calomel reference probe (Corning, Inc. Corning, NY) that were connected to a Fluke 73 Multimeter (John Fluke Manufacturing, Everett WA). A correction of +244 mV was added to redox measurements to compensate for the difference in redox potential between the calomel

probe and standard hydrogen reference electrode (Reddy and Delaune 2008). The Hach HQ 40d was calibrated monthly according to the manufacturer's instructions, while the redox probes were calibrated bi-monthly with 1 gram of 98% quinhydrone in a 100mL pH 7 solution. The experiment lasted for 50 days from 12 August to 1 October 2015.

The second experiment was a 3x3x4 factorial design with atrazine treatments and soil texture as the main effects. The soil texture of the experimental treatments was an organic-, clay-, or sand-dominated mixture based on rotating 65-30-5 percent proportions in which the dominant texture was 65%, the median component was 30%, and the third component comprised 5% of the mixture. However, the sand component was always the lowest fraction in the organic- and clay-dominated treatments (e.g., Clay-dominated texture: 65% clay, 30% organic, 5% sand; Organic-dominated texture: 65% organic peat, 30% clay, 5% sand) and the organic component comprised the median proportion in the sand- and clay-dominated treatments (i.e. 30%). Atrazine treatments, which were added monthly in a 1 L deionized water solution, were as follows: Control (0 µg L^{-1}), Low (1.0 µg L^{-1}), Medium (3.0 µg L^{-1}), and High (5.0 µg L^{-1}). There were four untreated controls with plants, four disturbed soil texture controls with no plants for each soil texture, and four deionized water disturbed controls. The disturbed controls were treated with a 3.0 µg L^{-1} atrazine solution on a monthly basis. Water levels were maintained 0.75 cm above the soil surface between treatments to ensure saturated soil conditions by adding 100–150 mL increments of deionized water to each unit. Soil temperature, pH, and redox potential were measured on a monthly basis as mentioned above. The experiment lasted for 204 days from 22 November 2015 until 15 June 2016.

Tensile Strength Testing

Only live root samples were used for tensile strength testing because of the short growing period, small belowground biomass, and the paucity of dead roots. In addition, the plant samples did not have sufficient time to produce fully developed fibrous root systems or to generate large numbers of dead roots via turnover. Consequently, tensile strength testing was conducted on live roots in only one of the four diameter size classes that were utilized by Hollis and Turner (2018). The small size class (0.5–1.0 mm) was selected for testing because of the high numbers of roots within this diameter range and the increased probability of conducting successful tensile strength tests. Six tests were conducted for every successful tensile strength test. A successful test consisted of root samples that failed between the supports of the test stand, whereas roots that failed at the point of contact on the supports were considered unsuccessful tests and the data were considered invalid. Live roots and rhizomes were differentiated from dead roots by their white, turgid, and translucent appearance whereas dead roots were dark and flaccid (Darby and Turner 2008). However, many live roots were stained by soil deposits. They were separated from dead roots by the presence of turgor, bifurcations of fine roots, and their ability to float. Five individual root metrics were measured: mass, length, diameter, cross-sectional area, and volume. Root length was measured with a Scale Master© Classic digital planimeter (Calculated Industries, Carson, Nevada USA) to the nearest 0.1 mm. The mean root diameter was measured to the nearest 0.1 mm with a Starrett digital IP67 micrometer. The measurements were taken at both ends and at the middle of each root and averaged. The cross-sectional area (mm^2) and volume (mm^3) were calculated from length and diameter measurements after tensile strength testing was performed. Fine root hairs of each test sample were trimmed to 0.16 cm (1/16 inch) with an X-acto© craft knife. Three roots were destructively sampled to determine the mass of the

remaining 0.16 cm projections, which was a correction factor that was subtracted from root mass. Root samples were weighed on a scale to estimate individual mass to the nearest 0.1 milligram (mg). A Mecmesin MultiTest 1d motorized stand (Mecmesin Limited; Sinfold, West Sussex, United Kingdom) was used to test tensile root strength in Newtons (N). Individual roots were secured to two support clamps that were perpendicular to the base of the test stand. The contact surfaces of the clamps provided an area of 1.25 x 2.50 cm and were lined with fine sandpaper to reduce or eliminate slippage. In addition, the support clamps were attached to a Mecmesin Basic Force Gage load meter, which was capable of measuring 1000 N of force with a precision of 0.1 N. The test stand was activated and the top support was pulled upward by a vertical hydraulic piston until the root exhibited structural failure. The load that induced failure at that point, or breaking force, was recorded as tensile strength. Leaf and root samples and porewater were sent to the LSU Agricultural Chemistry Laboratory to test for atrazine concentrations. The detection limit for leaf and root samples was 25 µg L^{-1}; however, the detection limit for porewater samples was 0.1 µg L^{-1}.

Statistical Analyses

In Experiment One, I conducted a one-way analysis of variance (ANOVA) in JMP v. 13 statistical software (SAS Cary, NC) to test for significant differences between the Control (0 µg L^{-1}) and the Low (0.5 µg L^{-1}), Medium (1.5 µg L^{-1}), and High (3.0 µg L^{-1}) atrazine treatments.

In Experiment Two, the differences in the mean tensile strength of roots by soil texture and atrazine treatment were detected using ANOVA in JMP v. 13. I tested for interactive effects by segregating the tensile root strength data of the levels of one main effect into subsets and then conducting one-way ANOVA of tensile root strength using each level of the other main effect. For instance, the tensile root strength data were divided by the three levels of the atrazine

main effect into High (5.0 µg L^{-1}), Medium (3.0 µg L^{-1}), and Low (1.0 µg L^{-1}) subsets and then one-way ANOVAs of tensile root strength were conducted for Organic, Clay, and Sand levels of the soil texture main effect (e.g. Tensile strength x Organic soil texture using the High atrazine data subset).

In both experiments, tests to determine any significant differences between the tensile root strength means used a Tukey-Kramer Honest Significant Difference (HSD) test. The data are reported as the mean ± 1 standard error of the mean (µ ± 1 SE) unless otherwise noted. Homoscedasticity and normality of residuals were determined with Brown-Forsythe and Shapiro-Wilk tests, respectively. Data that did not meet the assumptions of ANOVA were tested with a Welch's ANOVA and differences between the tensile strength means were determined using a Steel-Dwass nonparametric multiple comparison test. Interactive effects of treatment combinations were determined by using a Kolmogorov-Smirnov goodness-of-fit test to compare the data distribution of the combination with that of the strongest main effect of the treatment combination. Statistical significance among the soil temperature, redox potential, and pH parameter data were tested using a one-way ANOVA. All statistical tests were performed at a significance level of $p < 0.05$.

RESULTS

Experiment One: Tensile Root Strength

A one-way ANOVA produced no significant difference in tensile root strength between atrazine treatments and control (Fig. 3.1a; $F = 1.0024$, $p = 0.3934$). In addition, there was no significant difference in tensile root strength between the atrazine treatments. The tensile root strength grand mean between treatments and control was 4.6 ± 0.3 N. The soil temperature ranges for the low, medium, and high atrazine treatments in Experiment One were 23.9–34.8

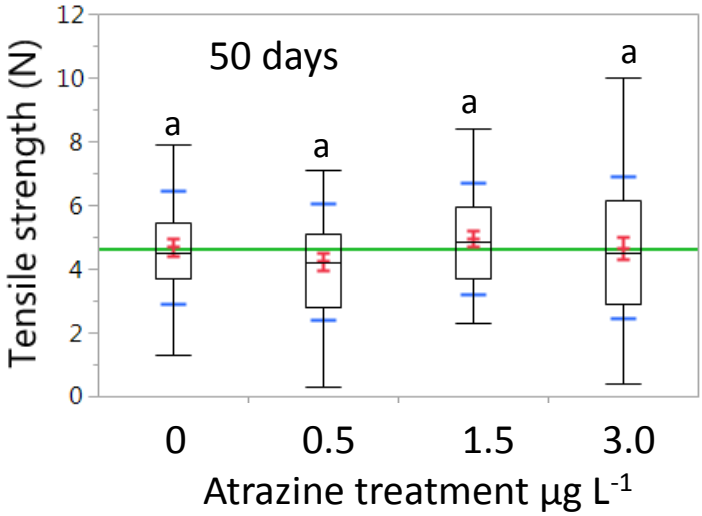
(27.7 ± 0.52 °C) 24.0–33.0 (27.6 ± 0.46 °C), and 24.0–34.9 (27.9 ± 0.54 °C), respectively (Table 3.1). The soil temperature of the control ranged from 23.4 to 34.3 (27.6 ± 0.53) and the mean air temperature within the greenhouse during the experiment was 27.6 ± 0.50 °C. The pH ranges for the low, medium, and high atrazine treatments were 6.9–7.2 (7.1 ± 0.02), 6.9–7.2 (7.0 ± 0.02), and 7.0–7.4 (7.1 ± 0.02), respectively (Table 3.1). The pH of the control ranged from 6.8 to 7.3 (7.0 ± 0.03). The redox potential ranges for the low, medium, and high atrazine treatments were –9.3 to 27.2 (8.3 ± 1.9 mV), –12.1 to 61.5 (12.7 ± 4.0 mV), and –29.2 to 3.1 (–9.2 ± 1.8 mV), respectively. The redox potential of the control ranged from –38.4 to 24.7 (–2.2 ± 3.1 mV).

Experiment Two: Tensile Root Strength

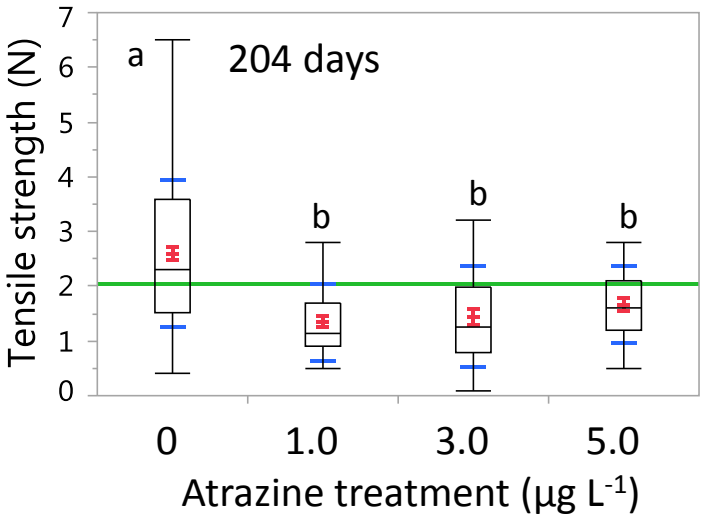
The results from a one-way ANOVA revealed significant differences in the tensile root strength between all atrazine treatments and Control (Fig. 3.1b; $F = 21.5$, $p < 0.0001$); however, there were no significant differences among the tensile root strength of the atrazine treatments. The grand tensile root strength mean was 2.04 ± 0.17 N and there were no significant differences in tensile strength among the three atrazine treatments.

The results from a one-way Welch's ANOVA also revealed that the tensile root strength of the three atrazine treatments in the organic, clay, and sand subsets were significantly different from the Control (Fig 3.2a-c; $F = 15.0$, $p < 0.0001$; $F = 4.5$, $p = 0.026$; $F = 15.2$, $p < 0.0001$). However, there were no significant differences in tensile root strength among the atrazine treatments, and the grand mean tensile root strengths for each atrazine-soil texture subset were 1.79 ± 0.14, 1.55 ± 0.15, and 1.76 ± 0.14 N in the Low, Medium, and High treatments, respectively.

The results of a one-way Welch's ANOVA revealed significant differences in tensile root strength between all soil texture treatments and among the soil texture Controls (Fig. 3.3, $F = 16.7$, $p < 0.0001$).



(a)



(b)

Fig. 3.1 Box-and-whisker plots of (a) a one-way ANOVA of tensile root strength with atrazine as the main effect for the first atrazine greenhouse experiment (b) One-way ANOVA of tensile root strength with atrazine as the main effect for the second atrazine greenhouse experiment. Tensile root strength in the control (0 µg L^{-1}) was significantly higher than in low (1.0 µg L^{-1}), medium (3.0 µg L^{-1}), and high (5.0 µg L^{-1}) atrazine treatments (F = 17.9, F = 16.4, F = 15.9, respectively; $p < 0.0001$). There was no significant difference between control and atrazine treatments or among atrazine treatments in Experiment One (p = 0.3934). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

However, the Organic (CO) and Sand (CS) Controls were significantly different from the Clay Control (CC) (2.90 ± 0.14 and 2.88 ± 0.14 N vs. 2.02 ± 0.14 N, respectively). The grand tensile root strength mean was 1.99 ± 0.16 N, and there were no significant differences among the tensile root strengths of the soil texture treatments.

Table 3.1 Results of a one-way ANOVA and summary of the soil parameter testing for atrazine Experiment One. Statistical significance between the means is indicated by values with different letters ($p < 0.05$)

Parameter	**Experimental Treatments**			
	Control	**Low**	**Medium**	**High**
Soil Temperature (°C)				
Mean	27.6	27.7	27.6	27.9
Min	23.4	23.9	24.0	24
Max	34.4	34.8	33.0	34.9
Standard Error	0.53	0.52	0.46	0.54
pH				
Mean	7.0	7.1	7.0	7.1
Min	6.8	6.9	6.9	7.0
Max	7.3	7.2	7.2	7.4
Standard Error	0.03	0.02	0.02	0.02
Redox Potential (mV)				
Mean	–2.2	8.3	12.7	–9.2
Min	–38.4	–9.3	–12.1	–29.2
Max	24.7	27.2	61.5	3.1
Standard Error	3.1	1.9	4.0	1.8

A one-way Welch's ANOVA of tensile root strength with soil texture as the main effect in the High atrazine treatment subset found significant differences in tensile root strength between the Organic and Sand Controls and all three soil texture treatments (Fig 3.4a; Table 3.2, 3.3; F =15.9, p <0.0001). However, the tensile root strength in the organic (2.90 ± 0.17 N) and sand (2.88 ± 0.17 N) Controls were statistically higher than in the Clay Control (2.02 ± 0.17 N). There were no significant differences in tensile root strength between the Clay Control and the Clay and Organic treatments ($p > 0.05$).

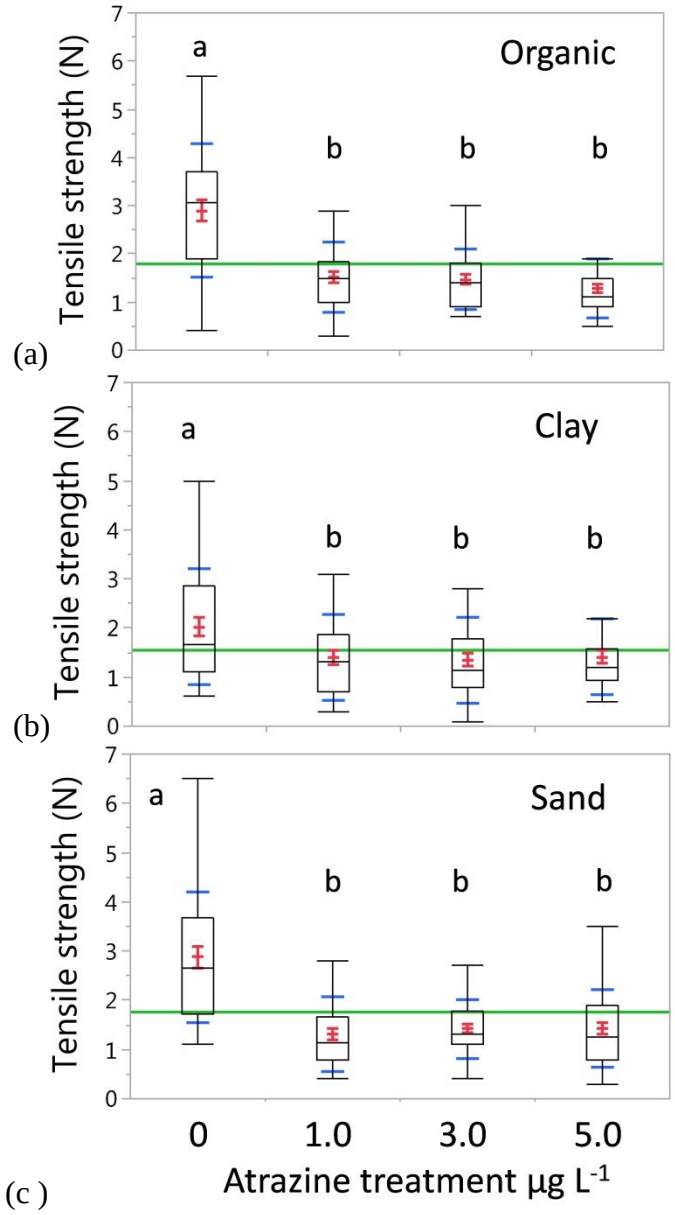


Fig. 3.2 Box-and-whisker plots of a one-way Welch's ANOVA of tensile root strength for (a) organic soil texture data subset (b) clay soil texture data subset (c) sand soil texture data subset with atrazine as the main effect for the second atrazine greenhouse experiment to test for interactive effects between atrazine and soil texture treatments. Tensile root strength in the Controls (0 µg L^{-1}) were significantly higher than in low (1.0 µg L^{-1}), medium (3.0 µg L^{-1}), and high (5.0 µg L^{-1}) atrazine treatments for organic, clay, and sand subsets (F = 15.0, F = 4.5, F = 15.2, respectively; p<0.0001). There were no significant differences between the atrazine treatments (p = 0.3934). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

However, the tensile root strength in the Clay control (2.02 ± 0.17 N) was significantly different from the Sand treatment (1.31 ± 0.17 N). The grand mean in the high atrazine subset was 2.0 ± 0.17 N. The results indicated that when the high atrazine treatment was combined with the sand soil texture, tensile root strength decreased by 55% versus the sand Control (i.e. from 2.88 N to 1.31 N) and 36% vs. the clay Control (i.e. from 2.02 N to 1.31 N).

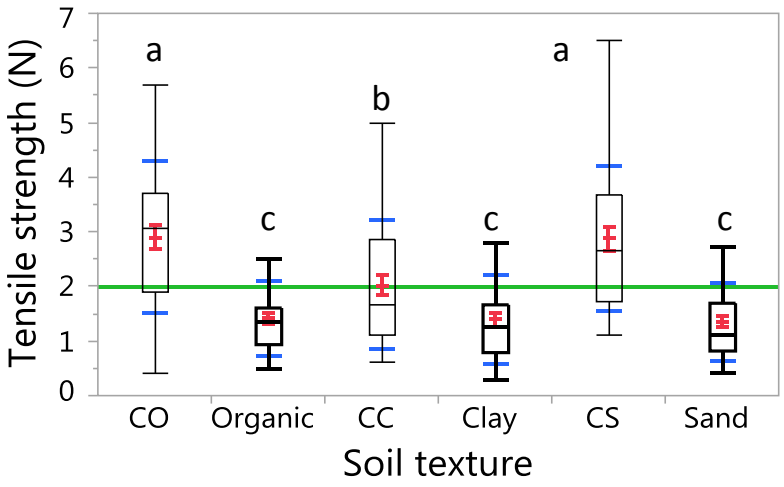


Fig. 3.3 Box-and-whisker plots of a one-way ANOVA of tensile root strength with soil texture as the main effect for the second 204-day atrazine greenhouse experiment. Tensile root strength in all Controls (CO = Control Organic, CC = Control Clay, CS = Control Sand) were significantly higher than all soil texture treatments ($F = 16.7$, $p < 0.0001$); however, CO and CS were significantly different from CC. There were no significant differences between the soil texture treatments ($p = 0.9988$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

In the Medium atrazine treatment subset, there were significant differences in tensile root strength between the Organic and Sand controls and all three soil texture treatments as well as the Clay Control (Fig 3.4b; Table 3.2, 3.3; $F = 16.4$, $p < 0.0001$). The grand mean in the Medium atrazine subset was 2.0 ± 0.17 N.

In the results for the Low atrazine treatment subset, a one-way Welch's ANOVA of the soil texture main effect found significant differences in tensile root strength between the Organic and Sand Controls and all three soil texture treatments (Fig 3.4c; Table 3.2, 3.3; $F = 17.9$, $p < 0.0001$). However, the Organic (2.90 ± 0.17 N) and Sand (2.88 ± 0.17 N) Controls were statistically different from the Clay control (2.02 ± 0.17 N). There were no significant differences between the Clay control and the Clay and Sand treatments ($p > 0.05$), but the Clay Control (2.02 ± 0.17 N) was significantly different from the Organic treatment (1.28 ± 0.17 N). The grand mean in the Low atrazine subset was 1.98 ± 0.17 N.

Table 3.2 Summary of one-way Welch's ANOVA tests of the tensile root strength response variable for the atrazine treatment and soil texture main effects and main effect subset (in parentheses) testing for interactive effects in Experiment Two. Statistical significance is indicated by *p*-values < 0.05

Source	[1]DFNum	[2]DFDen	F Ratio	*p*-value
Soil Texture	5	108.0	16.7	< 0.0001
Soil Texture (High)	5	108.2	15.9	< 0.0001
Soil Texture (Medium)	5	107.3	16.4	< 0.0001
Soil Texture (Low)	5	107.7	17.9	< 0.0001
Atrazine	3	104.4	21.5	< 0.0001
Atrazine (Organic)	3	84.8	15.0	< 0.0001
Atrazine (Clay)	3	85.9	4.5	0.0046
Atrazine (Sand)	3	84.5	15.2	< 0.0001

[1]Degrees of Freedom -Numerator; [2]Degrees of Freedom - Denominator

These results indicated that when the Low atrazine treatment was combined with the Organic soil texture, tensile root strength decreased by 57% compared to the tensile root strength in the Organic Control (i.e. from 2.90 N to 1.28 N) and 37% in the Clay Control (i.e. from 2.02 N to 1.28 N).

The results from a two-sample Kolmogorov-Smirnov goodness-of-fit test found no statistically significant interactive effects of atrazine and soil texture in the 18 soil texture-atrazine treatment combinations on the tensile root strength of *S.patens* ($p > 0.05$).

Experiment Two: Soil Parameters

The soil temperature in the experimental treatments ranged from 23.4 to 29.1 °C (Table 3.4; Appendix A, Fig. A1) with a mean of 25.5 (± 0.23 °C, SE), and there was less than a 1°C variation between the mean temperature for each soil texture. An ANOVA revealed no significant difference between the soil temperatures among the three soil textures (Table 3.4, $p > 0.05$). The soil texture experimental Controls exhibit a similar pattern as the experimental treatments and an ANOVA revealed no significant differences among the soil texture Controls and the disturbed Control (Table 3.4, $p > 0.05$).

The pH of the experimental treatments was acidic throughout the experiment and ranged from 5.7 in the Clay and Organic treatments to 6.2 in the Clay treatments (Table 3.4; Appendix A, Fig. A2). An analysis of variance revealed significant differences between the soil pH among the three soil textures (Table 3.2, $p < 0.05$). The pH of the Organic treatments remained consistently below 6.0, while the Clay and Sand treatments fluctuated above and below pH 6.0. Also, there were significant differences in soil pH among the soil texture Controls and the disturbed Controls (Table 3.4, $p < 0.05$).

The redox potential varied considerably between the experimental treatments throughout the duration of the experiment. An analysis of variance revealed significant differences between the soil redox potential among the three soil textures (Table 3.4; Appendix A, Fig. A3).

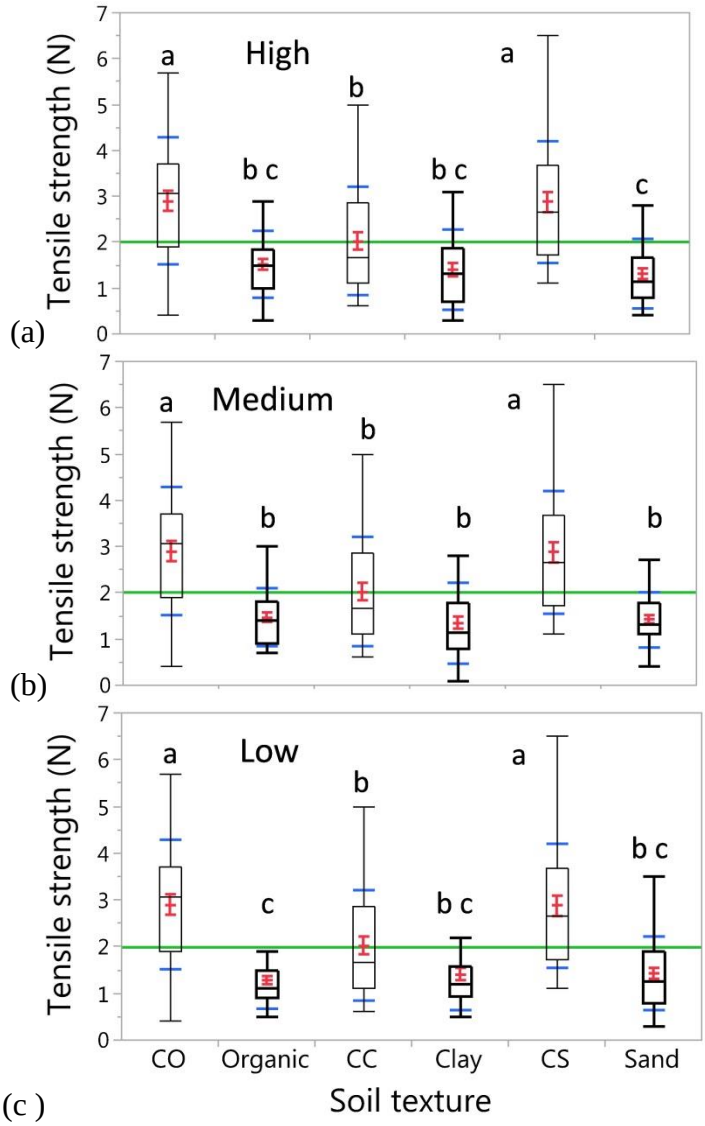


Fig 3.4 Box-and-whisker plots of a one-way Welch's ANOVA of tensile root strength for the (a) high atrazine data subset (b) medium atrazine data subset (c) low atrazine data subset with soil texture as the main effect for the second atrazine greenhouse experiment to test for interactive effects between atrazine and soil texture treatments. Tensile root strength in the organic and sand Controls (CO = Control Organic, CS = Control Sand) were significantly higher than in the organic, clay, and sand treatments in the low, medium, and high subsets (F = 17.9, F =16.4, F =15.9 respectively; $p < 0.0001$); however, CC = Control Clay, was significantly different from CO and CS and two treatments (See Table 3.3, $p < 0.05$). There were no significant differences between the atrazine treatments ($p = 0.3934$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red line represents the group mean and the two red lines above and below represents ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

Table 3.3 Summary statistics of the tensile root strength response variable for the atrazine treatment and soil texture main effects and main effect subset (in parentheses) testing for interactive effects in Experiment Two. Statistical significance is indicated by *p*-values < 0.05

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Atrazine	240	n/a	n/a	n/a	1.48	2.04	n/a	n/a	< 0.0001
Control	120	6.5	0.4	2.60	n/a	n/a	0.10	1.35	***
Low	40	4.0	0.5	1.35	n/a	n/a	0.17	0.70	< 0.0001
Medium	40	4.3	0.1	1.44	n/a	n/a	0.17	0.92	< 0.0001
High	40	3.9	0.5	1.66	n/a	n/a	0.17	0.69	< 0.0001
Atrazine (Organic)	160	n/a	n/a	n/a	1.42	1.79	n/a	n/a	< 0.0001
Control	40	5.7	0.4	2.90	n/a	n/a	0.14	1.39	***
Low	40	4.0	0.5	1.28	n/a	n/a	0.14	0.62	< 0.0001
Medium	40	3.2	0.7	1.47	n/a	n/a	0.14	0.63	< 0.0001
High	40	3.4	0.3	1.52	n/a	n/a	0.14	0.72	< 0.0001
Atrazine (Clay)	160	n/a	n/a	n/a	1.37	1.55	n/a	n/a	0.0265
Control	40	5.0	0.6	2.02	n/a	n/a	0.15	1.18	***
Low	40	3.9	0.5	1.41	n/a	n/a	0.15	0.78	0.0229
Medium	40	3.7	0.1	1.36	n/a	n/a	0.15	0.87	0.0102
High	40	4.2	0.3	1.40	n/a	n/a	0.15	0.88	0.02
Atrazine (Sand)	160	n/a	n/a	n/a	1.36	1.76	n/a	n/a	< 0.0001
Control	40	6.5	1.1	2.88	n/a	n/a	0.14	1.33	***
Low	40	3.5	0.3	1.43	n/a	n/a	0.14	0.78	< 0.0001
Medium	40	3.0	0.4	1.43	n/a	n/a	0.14	0.60	< 0.0001
High	40	3.9	0.4	1.31	n/a	n/a	0.14	0.77	< 0.0001

(Table 3.3 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Soil Texture	240	n/a	n/a	n/a	1.39	1.99	n/a	n/a	< 0.0001
Organic	40	4.3	0.5	1.42	n/a	n/a	0.16	0.69	< 0.0001
Control Organic (CO)	40	5.7	0.4	2.90	n/a	n/a	0.16	1.39	< 0.0001
Clay	40	4.2	0.3	1.39	n/a	n/a	0.16	0.82	< 0.05
Control Clay (CC)	40	5.0	0.6	2.02	n/a	n/a	0.16	1.18	< 0.05
Sand	40	3.9	0.4	1.35	n/a	n/a	0.16	0.72	< 0.0001
Control Sand (CS)	40	6.5	1.1	2.88	n/a	n/a	0.16	1.33	< 0.0001
Soil Texture (High)	240	n/a	n/a	n/a	1.41	2.00	n/a	n/a	< 0.0001
Organic	40	3.4	0.3	1.52	n/a	n/a	0.17	0.72	< 0.0001
Control Organic (CO)	40	5.7	0.4	2.90	n/a	n/a	0.17	1.39	< 0.0001
Clay	40	4.2	0.3	1.40	n/a	n/a	0.17	0.88	< 0.05
Control Clay (CC)	40	5.0	0.4	2.02	n/a	n/a	0.17	1.18	ns
Sand	40	3.9	0.4	1.31	n/a	n/a	0.17	0.77	< 0.0001
Control Sand (CS)	40	6.5	1.1	2.88	n/a	n/a	0.17	1.33	< 0.0001

(Table 3.3 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Soil Texture (Medium)	240	n/a	n/a	n/a	1.43	2.01	n/a	n/a	< 0.0001
Organic	40	3.2	0.7	1.47	n/a	n/a	0.17	0.63	< 0.0001
Control Organic (CO)	40	5.7	0.4	2.90	n/a	n/a	0.17	1.39	< 0.0001
Clay	40	3.7	0.1	1.36	n/a	n/a	0.17	0.87	< 0.05
Control Clay (CC)	40	5.0	0.6	2.02	n/a	n/a	0.17	1.18	ns
Sand	40	3.0	1.1	1.43	n/a	n/a	0.17	0.60	< 0.0001
Control Sand (CS)	40	6.5	0.4	2.88	n/a	n/a	0.17	1.33	< 0.0001
Soil Texture (Low)	240	n/a	n/a	n/a	1.35	1.98	n/a	n/a	< 0.0001
Organic	40	4.0	0.5	1.28	n/a	n/a	0.16	0.6	< 0.0001
Control Organic (CO)	40	5.7	0.4	2.90	n/a	n/a	0.16	1.39	< 0.0001
Clay	40	3.9	0.5	1.41	n/a	n/a	0.16	0.78	< 0.05
Control Clay (CC)	40	5.0	0.6	2.02	n/a	n/a	0.16	1.18	ns
Sand	40	3.5	0.3	1.43	n/a	n/a	0.16	0.78	< 0.0001
Control Sand (CS)	40	6.5	1.1	2.88	n/a	n/a	0.16	1.33	< 0.0001

The redox potentials of the Control treatments were similar in range and magnitude to the experimental treatments. An analysis of variance revealed significant differences in redox potential among the soil texture Controls and the disturbed Control (Table 3.4, $p < 0.05$).

Table 3.4 Results of a one-way ANOVA and summary of the soil parameter testing for soil texture-atrazine Experiment Two. Statistical significance between the means is indicated by values with different letters ($p < 0.05$)

Parameter	Experimental Treatments			Controls			
	Organic	**Clay**	**Sand**	**Organic**	**Clay**	**Sand**	**No Plant**
Soil Temperature (°C)							
Mean	25.4[a]	25.7[a]	25.7[a]	25.1[a]	25.5[a]	25.2[a]	25.7[a]
Min	23.4	23.4	23.6	23.4	23.6	23.4	23.8
Max	27.3	28.1	29.1	26.5	27.9	26.3	27.9
Standard Error	0.21	0.21	0.26	0.19	0.21	0.15	0.19
pH							
Mean	5.1[a]	6.0[bc]	5.9[bc]	5.0[a]	6.0[c]	6.1[c]	5.5[d]
Min	5.0	5.7	5.8	4.7	5.6	5.9	5.1
Max	5.5	6.2	6.0	5.6	6.2	6.1	5.9
Standard Error	0.02	0.02	0.01	0.04	0.02	0.01	0.04
Redox Potential (mV)							
Mean	108.0[a]	54.5[b]	58.8[b]	113.0[a]	56.0[b]	62.1[b]	85.3[c]
Min	77.6	24.3	38.5	87.5	20.7	28.5	72.7
Max	140.0	72.1	84.2	148.6	80.6	83.1	96.5
Standard Error	3.1	2.2	2.2	3.0	2.7	2.9	1.4

Atrazine Testing

Neither atrazine nor any of its primary metabolites were detected in either leaf or root samples from any of the Organic, Clay, or Sand experimental treatments. However, atrazine was detected in the soil porewater of the disturbed Controls (Control-No Plant, CNP) at a concentration of 0.28 µg L^{-1} and deethylatrazine (DEA) was detected at 0.1 µg L^{-1}. In addition, atrazine and DEA were detected in the deionized water Controls at mean concentrations of 6.96 and 1.60 µg L^{-1}, respectively.

DISCUSSION

How does atrazine (and its metabolites) affect the tensile root strength of an emergent macrophyte? The uptake of atrazine is affected by soil texture, pH, temperature, redox potential, and species-specific adaptations that may render the plant resistant to the herbicide. These factors will either constrain or enhance the plant's ability to assimilate any available atrazine. Atrazine inhibits photosynthesis by preventing the transfer of electrons from Photosystem II to Photosystem I, which disrupts the ability of the plant to fix carbon dioxide and produce the energy required for survival. As the leaves succumb to atrazine exposure, transpiration and stomatal conductance may be affected and the soil-plant-water continuum could break down. The loss of water potential may eventually affect the roots' ability to acquire water and nutrients from the soil. In addition, the loss of turgor pressure will directly affect the tensile strength and structure of the roots by changing the orientation of microfibrils within the cell walls (Niklas 1992). Therefore, the resultant loss of photosynthate due to the herbicide-induced disruption of photosynthesis, combined with the loss of water and nutrients, could have a negative effect on the physiology of the belowground biomass, which may cause a reduction in tensile root strength. Soil texture and atrazine concentration interaction decreased the tensile root strength of *S. patens* over a 6-month period. The results for each atrazine experiment are discussed next in terms of biomechanical properties as well as other influences on tensile root strength; atrazine-soil interactions and the effects of soil pH, temperature, and redox potential.

Experiment One

Weekly atrazine treatments did not produce significant effects on the tensile root strength of *S. patens* after a 50-day experiment. First, the difference in magnitude between the three atrazine doses was not statistically significant; 3.0 μg L^{-1} was no more effective than 1.5 μg L^{-1}.

As a result, there may have been less variation in the effects of the herbicide doses. I acknowledge that this may be a shortcoming of the study; however, *S. patens* may have exhibited tolerance to the doses of atrazine that were administered. The plant may have successfully metabolized the herbicide before it could harm the plant. Lytle and Lytle (1998) demonstrated that *S. alterniflora* could tolerate atrazine doses as high as 3 mg L^{-1} over a 5-week period. It is unknown if *S. patens* also possesses this ability, but the results of this study suggest that this species has some level of tolerance to atrazine exposure. The duration of the experiment as well as the amount and rate of atrazine exposure, may not have been sufficient to induce biomechanical changes within the plant that would be manifested as declining tensile root strength. Moreover, the soil was composed of 65% sphagnum peat and 30% clay that may have contributed to the adsorption of atrazine, which would have made it unavailable to the plants. However, the soil temperature could have inhibited atrazine adsorption as well. Singh and Cameotra (2013) reported that two agricultural soils with clay contents ranging from 25 to 37% adsorbed less atrazine at 35 °C than at temperatures at or below 25 °C. In addition, the soil pH frequently fluctuated above and below the neutral threshold, at which neither acidic nor alkaline conditions were prevalent enough to favor either adsorption or desorption of atrazine. Gu et al. (1992) reported no degradation of atrazine in three Virginia wetland soils regardless of soil texture, temperature, or anaerobic conditions. Conversely, Gu et al. (2003) found that 30% of atrazine remained in microcosms containing three Chinese soils under methanogenic conditions after 300 days. The resilience and resistance of *S. patens* may have been sufficient to withstand atrazine exposure at concentrations of 0.5–3.0 µg L^{-1} for 7 weeks, or metabolize the herbicide. Perhaps the organic-dominated soil texture, in concert with the clay component, may have rendered atrazine unavailable to the plant. Consequently, the belowground biomass may not have

been impaired by atrazine exposure, which resulted in no significant differences in tensile root strength between the atrazine treatments and Control.

Experiment Two

The results of tensile root strength tests revealed significant difference in tensile root strength between the three atrazine doses and Control, but there were no significant differences in tensile root strength among the three atrazine doses. In addition, there were significant differences between the soil texture-atrazine combination treatments and the soil texture Controls, as well as significant differences among the soil texture Controls. This suggests that there were attributes of each soil texture that altered the effects of atrazine on the tensile root strength of *S. patens*.

The tensile root strength of *S. patens* may have been affected by a combination of soil texture and root architecture attributes. Soil texture is the proportion of sand, silt, clay, and organic matter in the soil, which can affect the strength of a plant's anchorage in the soil. For instance, the grain sizes of soil components directly influence the formation of macro- and micropores in the soil. Coarse soil textures, such as those in the Sand and Organic treatments, were larger than the fine-grained materials such as silt and clay. Consequently, the coarse soil textures created macropores and held less water, whereas the fine soil textures created micropores and generally held more water because of the hydrogen bonding of water molecules to soil colloids, reduced percolation, or both. These soil pores influenced water flow, drainage, and water-holding capacity. The root architecture enhanced soil saturation by providing conduits of water percolation along root channels. In addition, the soil conditions in the rhizosphere promoted biogeochemical reactions due to radial oxygen loss and organic exudate deposits from the roots. The presence of saturated soil conditions alter redox potential and facilitate numerous

biogeochemical reactions that have significant implications for wetland ecosystems, such as nutrient cycling, microbial community composition, and the mitigation of harmful effects of phytotoxins and xenobiotics. In order for atrazine to affect tensile root strength, the herbicide must first be assimilated by the plant and plant uptake of the herbicide is dependent upon the environmental conditions in the rhizosphere.

Organic matter, in both solid and dissolved forms, has a great affinity for atrazine. The organic-dominated soils had a greater capacity to form macropores and sequester atrazine in a soil porewater solution. However, it is also likely that dissolved organic matter may have been present in the soil porewater, which could have facilitated atrazine adsorption. As a result, atrazine availability to plant uptake may have been affected by the size of the macropores in the organic experimental treatments. The mean tensile root strength was higher in the Organic treatments (1.79 ± 0.11 N) than in the Clay treatments (1.54 ± 0.11), while the mean tensile root strength in the Sand and Organic treatments varied by only 0.03 ± 0.14 N. This suggests that less atrazine was available in the Organic and Sand treatments and that more atrazine was available in and assimilated by the plants in the Clay treatments. Fine roots and root hairs can either senesce and become part of the exudates that are shed by the plant, or they may be physically dislodged by soil friction due to root growth, which contributes to the organic deposits in the soil that may serve as electron donors in redox reactions or become a binding mechanism for atrazine. Laird and Koskinen (2008) have stated that water molecules can outcompete atrazine molecules in bonding to variable charged surfaces in aqueous systems. Therefore, the impact of DOM on soil sorption and subsequent transport may be dependent on the intrinsic nature of the solute, soil, and DOM, water quality, and the competition among the solid and solution fractions of DOM and the soil (Seol and Lee 2000). Also, the nature of the microbial communities within

the sand and organic treatments may have been different, which could have affected atrazine transformation and mineralization. Furthermore, the rates of adsorption and desorption in the Organic treatments may have varied considerably. However, Chung et al (1996) cautioned that the heterogeneous nature of humic substances in organic matter can radically change adsorption and desorption dynamics. The Freundlich equation has been used in many studies for both adsorption and desorption to model the atrazine distribution between the solid and solution phases of the soil (Clay and Koskinen 1990a). The process in which the desorption isotherm may not be predicted accurately by the adsorption isotherm is referred to as hysteresis. Factors that may induce hysteresis include differential rates of adsorption and desorption (Kan et al. 1994, Singh and Cameotra 2013), the solubility of the herbicide, the nonattainment of equilibrium during either adsorption or desorption (Clay and Koskinen 1990a), irreversible binding of the herbicide to the soil (Calvert 1989), or loss of the compound due to degradation, transformation, or volatilization (Clay and Koskinen 1990b).

However, sandy soils can form larger micro- and macropores than fine-textured soils and hold larger volumes of soil porewater that can contain a mixture of DOM and clay colloids. Sand has a low adsorption affinity for atrazine. The inherent nature of sandy soils such as rapid infiltration, percolation, and drainage of water as well as small organic and clay fractions can limit their ability to curtail the transportation and degradation of herbicides (Seybold et al. 1994). The potential for macropore formation in sandy soils creates conditions for larger volumes of porewater in which atrazine may remain in solution. In addition, the dearth of organic matter and clay fractions would increase the availability of the herbicide to plants. Therefore, the soil texture in the Sand treatments and the possible adsorption of atrazine in the organic treatments could have facilitated similar tensile root strength results because of similar soil porewater

concentrations, which may have occurred because of plant absorption of the same general amount of atrazine. Turner and Dickens (1987) recorded reductions of the tensile strength of *E. ophiuroides* that had been established in a Dothan sandy loam (fine-loamy, siliceous, thermic Plinthic Paleudult). The Dothan series consists of very deep, well drained soils that contain 50–90% of sand in the A horizon and have a weak, granular, and very friable structure (USDA 2017a, NRCS 2017). They found that the tensile strength of the *E. ophiuroides* sod decreased in a linear fashion as atrazine application rates increased. These results suggest that sandy soils with low organic and clay fractions can prolong the availability of atrazine in the rhizosphere and eventually lead to a reduction of the tensile strength of the belowground biomass.

On the other hand, an increase in the clay content of a soil, along with a concomitant decrease in organic matter, may result in greater adsorption of atrazine. In addition, an increase in the clay fraction of the soil may result in a decrease of the mobility of the herbicide. However, the mean tensile root strength in the Clay treatments was lower than in either the organic or sand treatments. The decrease in the tensile root strength of the Clay treatments suggests that a greater amount of atrazine may have been assimilated by the plants than in the Organic or Sand treatments. Turner and Dickens (1987) recorded reductions of the tensile strength of *E. ophiuroides* sod that had been established in a Gilead sandy loam (clayey, kaolinitic, thermic Aquic Hapludult) and a Leaf silt loam (clayey, mixed, thermic Typic Albaquult). The Gilead series consists of very deep, moderately well drained, firm, clayey soils in the upper Coastal Plain with moderately slow or slow permeability (USDA 2017b). The Leaf series consists of very deep, poorly drained, very slowly permeable soils that formed in clayey alluvial and fluvial sediments (USDA 2017c). In both soil series, the clay content increases with depth (USDA 2017b, 2017c; NRCS 2017). Turner and Dickens (1987) found that the tensile strength of the *E.*

ophiuroides sod in these soils also decreased in a linear fashion as atrazine application rates increased. They suggest that the reduction in tensile strength may have been the result of an accumulation of atrazine in the soil. However, I agree with Turner and Dickens (1987) that the reduction in tensile strength may have been caused by atrazine, but I think that it was more likely that the atrazine reduced the tensile root strength because it was more available to plant absorption. In addition, there may have been weak adsorption and/or variation in the adsorption and desorption rates of atrazine, which may have produced hysteresis. The Clay treatments in this study may have exhibited a similar phenomenon because these treatments also contained an organic matter fraction (30%), which, in concert with the dominant clay component (65%), should have greatly increased the probability of atrazine adsorption to the soil. However, the effectiveness of the clay fraction to immobilize the herbicide may be influenced by the soil organic matter fraction, which has a greater affinity for atrazine. In addition, clayey soil particles can form complexes with organic matter, which can reduce herbicide adsorption (Clay and Koskinen 1990a). An increase in the soluble organic matter could have driven the increase in the insoluble organic fraction, which may have increased the mobility of the herbicide. Atrazine that is adsorbed onto dissolved organic solutes may be transported with the soil porewater solution to other areas or deeper into the soil profile by eluviation. On the other hand, the uptake of atrazine is dependent upon other factors besides clay and organic matter content. For instance, the water-holding capacity of clay-dominated soils can alter the redox potential, which would entrain other processes that could affect the assimilation of atrazine, such as decomposition of atrazine, its transformation into its primary metabolites, and the pH of the soil porewater.

Soil Texture-Atrazine Dynamics

The effects of various soil textures and atrazine exposure on the tensile root strength of *S. patens* depends upon 1) the availability of the herbicide to the plant 2) the plant absorption of the herbicide 3) interaction between the soil environment, plant attributes, and the fate, behavior, and concentration of the herbicide, which will determine its mobility, degradation, and/or transformation. The soil texture-atrazine treatment combination reduced the tensile root strength of *S. patens* by 31–57% in the soil texture subsets and by 29–56% in the atrazine subsets. Therefore, I will discuss the results of the tensile strength tests taking into consideration the parameter measurements to ascertain the nature of soil texture and atrazine dynamics on the tensile root strength of *S. patens*.

Numerous researchers have reported that organic matter, particularly humic substances, can immobilize atrazine. However, the atrazine dose that was as low as 1.0 µg L^{-1} ('Low' dose, Experiment 2) that was applied monthly, appears to have been a sufficient amount to weaken the tensile root strength of *S. patens* despite the high percentage of organic matter in the soil. The Low dose produced the same effect (i.e., similar mean tensile root strength) as the High and Medium dose. One explanation for this phenomenon could be that 1.0 µg L^{-1} is the lowest effective dose, or Lowest Observed Adverse Effect Concentration (LOAEC); whereas no additional effects were initiated by the higher doses because they did not meet an unknown threshold of a higher concentration effect. However, the herbicide had to be absorbed in order to negatively impact the plant.

The primary influence of soil texture on tensile root strength is its effect on the availability of the herbicide for plant absorption. The soil of the Organic treatments was comprised of 65 % Sphagnum peat, which was nearly 3 times the amount of the organic matter

fraction that was utilized by others. Before the experiments began, the 1.0 µg L^{-1} monthly doses of atrazine were not expected to produce any discernible effects because of the affinity of organic matter for atrazine. However, the mean tensile root strength in the Organic treatments was not significantly different from that in the either the Clay or Sand treatments. In addition, the soil pH in the Organic treatments remained moderately acidic and the redox potentials were moderately anaerobic. These three conditions (High organic matter, low pH, and low redox potentials) were 'ideal' for atrazine adsorption. In addition, the soil temperature in the Organic treatments ranged from 23.4 to 28.1 °C, which was within the temperature range reported by McGlamery and Slife (1966) as conducive to atrazine adsorption. They observed that atrazine adsorption to humic acid was greater at 40 °C than at 0.5 °C. Conversely, Harris and Warren (1964) found no significant difference in atrazine adsorption onto an organic soil at either 50 or 0 °C. Laird and Koskinen (2008) reviewed numerous atrazine studies and concluded that soil temperature could increase, decrease, or have no effect on atrazine adsorption due to the high number of permutations of soil component combinations.

There are several factors that could interfere with the affinity of organic matter for atrazine and inhibit adsorption. For instance, temperature can affect atrazine solubility and once in solution, the herbicide may be physically separated from the solid organic fraction of the soil. Pillai et al. (1977) reported that during the first 48 hours of their experiment, 90% of atrazine that had been absorbed by *S. alterniflora*, was present in the shoots. Cejudo-Espinoza et al. (2009) observed that atrazine accumulated in the roots of three emergent macrophytes in less than 10 minutes. Also, they found that there were two stages of plant uptake of atrazine as reported previously by Collander (1960) and Hance (1988): a rapid initial stage, primarily driven by interstitial diffusion, followed by a slower second stage facilitated by membrane transport. On

the other hand, the atrazine in solution would be available for complexation with dissolved organic matter, which would increase its mobility. Another reason for the possible lack of organic matter immobilization of atrazine was that the herbicide initially adsorbed to the organic fraction, but desorbed from the soil at a later period. As a result of the hysteresis effect, the rate of atrazine desorption could have exceeded the rate of adsorption. Furthermore, the composition of the organic peat may have had a lower affinity for atrazine. Laird and Koskinen (2008) reported that humic substances are highly heterogeneous in nature and that assumptions cannot be made about their interaction with atrazine.

The adsorbed parent compound atrazine could also have been transformed to its primary metabolites before being absorbed by the plants. The atrazine metabolites deethylatrazine (DEA) and deisopropylatrazine (DIA) were detected at 0.28 μg L^{-1} in the soil porewater and both metabolites have been reported to be as phytotoxic as the parent atrazine compound (Belluck et al. 1991, Meakins et al. 1995). Perhaps there were even negative effects of atrazine and its two primary metabolites that could have increased the toxic effect on the plants. Also, the alternate electron acceptors within the soil could have generated additional effects by the soil texture. The redox potential range of the Organic treatments was conducive for iron, manganese, and nitrate to be utilized by microbes. As a result, the tensile root strength of *S. patens* may have been weakened by the demand for carbon as an electron donor. When this is combined with the possible effects of the free radicals, root degradation may have been increased. On the other hand, there was no reduction of tensile root strength in the Experiment 1 treatments, which had the same soil composition as the Organic treatments in Experiment 2 and therefore may have had the same ions available for electron acceptors. However, the composition of the microbial communities had not been determined; therefore it is unknown whether or not microbial activity

contributed to tensile root strength reduction. A temporal component may have been a factor in the plants' response to atrazine treatments. In Experiment 1, the High atrazine dose (3.0 μg L^{-1}) had no significant effect on tensile root strength after 50 days. Conversely, the *Low* atrazine dose in Experiment 2 *did* significantly affect tensile root strength after 200 days. Perhaps the effects of the herbicide at that concentration began to overwhelm the plant's ability to mitigate the effects of the herbicide. Also, it may have taken a longer period of time for any negative effects of atrazine to manifest themselves as reduced tensile root strength. The effects of the soil conditions and atrazine doses may have curtailed the differences in the three soil textures to produce similar responses in tensile root strength.

Soils with high percentage of clay also have a similar affinity for atrazine, but they may not adsorb the herbicide as readily or as strongly as organic soils. Nevertheless, the Clay treatments were comprised of 65% clay and 30% organic peat, which should have greatly increased their ability to immobilize atrazine. The pH of the Clay treatments ranged from 5.7 to 6.2, which was conducive to atrazine adsorption by the soil. Therefore, given the pH levels and the affinity of organic matter and clay for atrazine, the 1.0 μg L^{-1} monthly doses of atrazine should have been intercepted by the soil. However, the mean tensile strength of the Clay treatments was not significantly different from that of either the Sand or Organic treatments.

Unlike the Organic and Sand treatments, the Clay treatments appeared to contain considerable amounts of metal cations, especially iron. The roots and root channels were coated with a reddish orange residue, which is usually indicative of oxidized iron deposits. Also, these redoximorphic features revealed the status of the redox potential in the soil. Pockets of redoximorphic features within the soil matrix are an indication of oxidized soil within the larger area of reduced soil and numerous aerobic-anaerobic interfaces. Atrazine is more easily degraded

and transformed under aerobic conditions than under anaerobic conditions. Consequently, the oxidized rhizosphere could have changed the dynamics of atrazine adsorption-desorption. Iron is also capable of forming complexes with organic matter, and these complexes could have locked up cation exchange sites on the organic molecules. In addition, Laird et al. (1994) reported that clay soil particles with Fe- and Al- oxyhydroxide coatings reduced the affinity of mineral surfaces for atrazine. With iron (and perhaps aluminum, calcium, and manganese) occupying adsorption sites, the atrazine molecules could have remained free or in solution and available for plant absorption. However, the soil pH and soil temperature may have complicated the fate of atrazine. The acidic conditions may have facilitated greater atrazine adsorption, but the increased temperature, according to Harris and Warren (1964), would have reduced absorption. Also, the soil temperature could have increased the activity of the microbial community.

Sharpe et al. (1989) reported a loss in tensile strength of *Cynodon dactylon* (Bermudagrass) sod that had been cultivated in Dothan loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudults). They found that the tensile strength was 50% lower in the eight weeks after treatment (8 WAT) of *C. dactylon* sod than two weeks after treatment (2 WAT). Dothan loamy sand is comprised of >85% sand in the A and E horizons (USDA 2017a); consequently, limited quantities of organic matter, silt, and clay are available for herbicide adsorption. Therefore, it would seem that soils with a considerable sand fraction are less likely to immobilize atrazine without organic matter, clay, and silt. Under these conditions, atrazine would be more available for plant absorption, which should theoretically cause a greater reduction in tensile root strength than in the Clay and Organic treatments. However, the coarse texture of the Sand treatments may contain more macropores, which would place the atrazine in solution along with dissolved organic matter; a condition that would reduce plant absorption. But

the acidic pH (5.7–6.1) and anaerobic redox potentials (+50 to +120 mV) that were measured in the Organic treatments were more favorable for adsorption. As a result, the atrazine that was in solution may have overcome the acidic and anaerobic conditions to affect the tensile root strength of the plants. On the other hand, the Sand treatments also exhibited redoximorphic features that were indicative of an oxidized rhizosphere. The roots from these treatments appeared to be coated with ferric iron oxide, which can affect root absorption of nutrients and other compounds. The oxidized rhizosphere can also create conditions for the aerobic decomposition and/or transformation of atrazine. Therefore, it may have been possible that the plants absorbed atrazine metabolites that eventually reduced tensile root strength, while the parent compound was bound to DOM, adsorbed to clay or organic particles, or inhibited by iron oxide plaque on the roots.

CONCLUSIONS

The effect of atrazine exposure on *S. patens* was dependent upon plant absorption of the herbicide and the components of the soil texture. Soil texture may cause either positive or negative feedbacks, depending upon environmental factors such as soil temperature, pH, and redox potential. This may explain the effects of the soil texture-atrazine treatment combinations on the tensile root strength of *S. patens*, which was affected by exposure to atrazine doses greater than or equal to 1 μg L^{-1} in sand-, clay-, and organic-dominated soils. In addition, the structure and type of soil components can have a significant effect on herbicide adsorption. The numerous potential permutations of type of clay, silt, and organic matter can confound the conclusions of previous studies, as demonstrated by the range of results concerning soil temperature. The tensile root strength of the experimental treatments was nearly 50% lower than that of the Controls. Also, there was no significant difference in tensile root strength among either the soil textures or

doses of atrazine. The reduction in tensile root strength suggests that the herbicide was translocated throughout the plant via the phloem after initial absorption and transport via the xylem. Atrazine did not appear to undergo significant photodegradation. In addition, the detection of the primary metabolites DEA and DIA in the soil porewater suggest that these compounds may have induced an additional effect on tensile root strength. The tensile root strength may have declined due to a reduction in photosynthetic rates that curtailed the plants' ability to meet carbon fixation demands for maintenance and growth. In addition, the herbicide may have generated free radicals that induced oxidative stress by attacking cells and cell walls. If these radicals were transported throughout the plant, then these radicals could have weakened the structural integrity of the roots. The lack of visible injury to the plants suggests that *S. patens* possesses some tolerance to atrazine exposure, but the reduction in tensile root strength is an indication that the plants did not escape unscathed. More importantly, this study has indicated that the LOAEC of atrazine for *S. patens* may be much lower than previously observed for other species of emergent macrophytes. The Low dose for these experiments was well below the ambient levels of atrazine that have been recorded in surface water stations on the Mississippi River (Welch et al. 2014). When transported in surface conveyances, atrazine may not be subjected to photodegradation because of its molecular structure, the turbidity of the water, and possible adsorption to suspended sediment. In addition, I am not aware of any monitoring of soil porewater atrazine concentrations, which are far more relevant to the health of coastal marshes than surface water measurements. Many researchers have demonstrated that atrazine does not bioaccumulate in living organisms; however, the herbicide is prone to hysteresis in organic matter mediums. In addition, not much is known about the dynamics of adsorption and desorption in wetlands that possess conditions to act as a sink for atrazine. In the case of

Louisiana and the Mississippi River Delta, the coastal wetlands are exposed to high fluxes of atrazine inputs from agricultural fields that are adjacent to the estuaries. These fluxes, although infrequent, are an order of magnitude greater than the atrazine concentrations that were used in this study. Consequently, these 'secondary sources' of atrazine may have a considerable additive effect with the 'ambient' levels of atrazine in the major tributaries and distributaries of the Mississippi River. The relative sea level is rising, and there are concerns about more frequent occurrences of tropical cyclones. It is important to understand factors that compromise tensile root strength in order to protect the sustainability of these ecosystems. The results of this study indicate that extensive field experiments are needed to ascertain the effect of atrazine on the tensile root strength of *S. patens* and other coastal emergent macrophytes that play a pivotal role in reducing and/or preventing coastal land loss in Louisiana and elsewhere.

REFERENCES

Belluck DA, Benjamin SL, Dawson T (1991) Groundwater contamination by atrazine and its metabolites *In*: Pesticide Transformation Products pp 254-273 DOI:10.1021/bk-1991-0459.ch018

Bodker JE, Turner RE, Tweel A, Schulz C, Swarzenski C (2015) Nutrient-enhanced decomposition of plant biomass in a freshwater wetland. Aquatic Botany 127:44-52

Borggaard OK, Streibig JC (1988). Atrazine adsorption by some soil samples in relation to their constituents. Acta Agriculturae Scandinavica 38:293–301

Brady NC, Weil RR (2002) The Nature and Properties of Soils, 13th edition. Prentice Hall Upper Saddle River, New Jersey 960 pp.

Calvert R (1989) Adsorption of organic chemicals in soils. Environmental Health Perspectives 83:145-177

Cejudo-Espinosa E, Ramos-Valdivia AC, Esparza-Garcia F, Moreno-Casasola P, Rodriguez-Vazquez R (2009) Short-term accumulation of atrazine by three plants from a wetland model system. Archives of Environmental Contaminant Toxicology 56:201–208

Chabreck RH (1972) Vegetation, Water and Soil Characteristics of the Louisiana Coastal Region. Louisiana State University Agricultural Experiment Station Bulletin No. 664, 72 pp.

Chung KH, Ro KS, Roy D (1996) Fate and enhancement of atrazine biotransformation in anaerobic wetland sediment. Water Resources 30(2):341-346

Clay SA, Koskinen WC (1990a) Adsorption and desorption of atrazine, hydroxyatrazine, and s-glutathione atrazine on two soils. Weed Science 38(3):262-266

Clay SA, Koskinen WC (1990b) Characterization of alachlor and atrazine desorption from soils. Weed Science 38(1):74-80

Collander R (1960) Cell membranes: their resistance to penetration and their capacity for transport. *In*: F Steward (ed.) Plant Physiology: A Treatise, Volume II. Academic Press, New York, 758 pp.

Darby, FA, Turner RE (2008) Effects of eutrophication on salt marsh roots, rhizomes, and soils. Marine Ecology Progress Series 363:63-70

Frissel, MJ (1961) The adsorption of some organic compounds, especially herbicides, on clay minerals. Verslagen Van Land-bouwkundige Onderzockingen NR. 67.3, Wageningen, Holland

Goolsby DA, Battaglin WA, Thurman EM (1993) Occurrence and Transport of Agricultural Chemicals in the Mississippi River Basin, July through August 1993. U.S. Geological Survey Circular 1120-C, 22 pp.

Gu JD, Berry DF, Taraban RH, Martens DC, Walker, Jr. HL, Edmonds WJ (1992) Biodegradability of Atrazine, Cyanazine, and Dicamba in Wetland Soils. Virginia Polytechnic Institute and State University, Virginia Water Resources Research Center Bulletin 172, 45 pp.

Gu JD, Fan Y, Gu JD (2003) Biodegradability of atrazine, cyanazine and dicamba under methanogenic condition in three soils of China. Chemosphere 52:1515–1521

Hance R (1988) Adsorption and bioavailability. In: Grover R (ed.) Environmental Chemistry of Herbicides. CRC Press, Boca Raton, FL, p 216

Harris CI, Warren GF (1964) Adsorption and desorption of herbicides by soil. Weeds 12 (2):120-126

Hayes MHB (1970). Adsorption of triazine herbicides on soil organic matter, including a short review on soil organic matter chemistry. *In*: FA Gunther and JD Gunther (eds.), The Triazine Herbicides: Residue Reviews 32. New York, NY: Springer-Verlag, pp. 131–174

Hollis LO, Turner RE (2018) The tensile root strength of five emergent coastal macrophytes. Aquatic Botany 146:39-47

Kan AT, Fu G, Tomson MB (1994) Adsorption/desorption hysteresis in organic pollutant and soil/sediment interaction. Environmental Science Technology 28:59-67

Laird DA, Yen PY, Koskinen WC, Steinhelmer PR, Dowdy RH (1994) Sorption of atrazine on soil clay components. Environmental Science Technology 28:1054-1061

Laird DA, Koskinen WC (2008) Triazine Soil Interactions *In:* H Lebaron, J Macfarlane, O Burnside (eds.) The Triazine Herbicides: 50 years Revolutionizing Agriculture. Elsevier, San Diego, CA pp 275-300

Larsen L, Jørgenson C, Aamand J (2001) Potential mineralization of four herbicides in a groundwater–fed wetland area. Journal of Environmental Quality 30:24–30

Lee KE, Huggins DG, Thurman EM (1995). Proceedings of the Versatility of Wetlands in the Agricultural Landscape Conference: Hyatt Regency, Tampa, Florida, September 17-20, 1995. St. Joseph, MI: American Society of Agricultural Engineers

Lytle JS, Lytle TF (1998) Atrazine effects on estuarine macrophytes *Spartina alterniflora* and *Juncus roemerianus*. Environmental Toxicology and Chemistry 17(10):1972–1978

McCormick LL, Hiltbold AE (1966) Microbiological decomposition of atrazine and diuron in soil. Weeds (14)1:77-82

McGlamery MD, Slife FW (1966) The adsorption and desorption of atrazine as affected by pH, temperature, and concentration. Weeds 14(3):237-239

Meakins NC, Bubb JM, Lester JM (1995) The mobility, partitioning and degradation of atrazine and simazine in the salt marsh environment. Marine Pollution Bulletin, (30)12:812-819

Mersie W, Seybold C (1996) Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine, and hydroxyatrazine on Levy wetland soil. Journal of Agricultural and Food Chemistry 44:1925-1929

Natural Resources Conservation Service (NRCS) (2017) Soil texture calculator. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167 Accessed online 15 June 2017

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago 608 pp.

Niklas KJ, Spatz HC (2012) Plant Physics. University of Chicago Press, Chicago 426 pp.

Pillai CGP, Weete JD, Davis DE (1977) Metabolism of atrazine by *Spartina alterniflora*: 1. Chloroform-soluble metabolites. Journal of Agricultural and Food Chemistry 25(4):852-855

Reddy KR, Delaune RD (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, New York 774 pp.

Senesi N, Testini C (1982). Adsorption of some nitrogenated herbicides by soil humic acids. Soil Science 130:314–320

Seol Y, Lee LS (2000) Effect of dissolved organic matter in treated effluents on sorption of atrazine and prometryn by soils. Soil Science Society of America Journal 64:1976–1983

Seybold CA, McSweeney K, Lowery B (1994) Atrazine adsorption in sandy soils of Wisconsin. Journal of Environmental. Quality 23:1291-1297

Sharpe SS, Dickens R, Turner DL (1989) Herbicide effects on tensile strength and rooting of Bermudagrass (*Cynodon dactylon*) sod. Weed Technology 3(2):353-357

Singh AK, Cameotra SS (2013) Adsorption and desorption behavior of chlorotriazine herbicides in the agricultural soils. Journal of Petroleum and Environmental Biotechnology (4)5:1-6

Stevenson FJ (1972) Role and function of humus in soil with emphasis on adsorption of herbicides and chelation of micronutrients. BioScience 22(11):643-650

Turner DL, Dickens R (1987) Atrazine effects on tensile strength of Centipedegrass sod. Agronomy Journal 79:39-42

Turner DL, Sharpe SS, Dickens R (1990) Herbicide effects on tensile strength and rooting of Centipedegrass sod. Horticultural Science 25(5):541-544

Turner RE (2011) Beneath the salt marsh canopy: Loss of soil strength with increasing nutrient loads. Estuaries and Coasts 33:1084-1093. doi: 10.1007/s12237-010-9341-y

United States Department of Agriculture (USDA) (2017a) Dothan soil series. https://soilseries.sc.egov.usda.gov/OSD_Docs/D/DOTHAN.html Accessed online 15 June 2017

United States Department of Agriculture (USDA) (2017b) Gilead soil series. https://soilseries.sc.egov.usda.gov/OSD_Docs/G/GILEAD.html Accessed online 15 June 2017

United States Department of Agriculture (USDA) (2017c) Leaf soil series. https://soilseries.sc.egov.usda.gov/OSD_Docs/L/LEAF.html Accessed online 15 June 2017

United States Environmental Protection Agency (USEPA) (2016) Refined Ecological Risk Assessment for Atrazine. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington D.C.

Welch HL, Coupe RH, Aulenbach BT (2014) Concentrations and transport of suspended sediment, nutrients, and pesticides in the Lower Mississippi-Atchafalaya River subbasin during the 2011 Mississippi River flood, April through July. US Geological Survey Scientific Investigations Report 2014–5100, 44 pp. http://dx.doi/10.3133/sir20145100

Weber JB, Weed SB, Ward TM (1969) Adsorption of s-Triazines by soil organic matter. Weed Science 17(4):417-421

CHAPTER 4
THE TENSILE ROOT STRENGTH OF *SPARTINA PATENS*: RESPONSE TO ATRAZINE EXPOSURE AND NUTRIENT ADDITION

INTRODUCTION

Wetlands may be de facto receiving basins for surface and subsurface flow because of their hydrogeomorphic position in the landscape. The hydropattern of these hydrologic inputs can influence the water quality and biogeochemical processes in wetlands and adjacent ecosystems. Nonpoint pollution sources, in particular, bring excess nutrient loads and herbicides into wetlands as a consequence of the increased uses of reactive nitrogen and phosphorus for food, fuel, and fiber for the human population (Galloway et al. 2008, Rabalais 2009, Ruddiman 2013). These anthropogenic sources increase eutrophication frequency and severity, which (Nixon 1995, Rabalais 2009) sometimes creates the formation of hypoxic or 'dead zones' in nearshore marine or estuarine environments (D'Elia et al. 1986, Dortch et al. 1994, Turner et al. 2008, Rabalais et al. 2001, Rabalais 2009), alters nutrient cycles (Justić et al. 1995, Justić et al. 1997, Turner and Rabalais 1994, Turner et al. 1998, Rabalais et al. 1996), and disrupts tropic dynamics in food webs (Reish et al. 1980, Conley et al. 1993, Justić et al. 2002). The influxes of excess nutrient loads provide numerous alternate electron acceptors for oxidation-reduction (hereafter, redox) reactions, which are utilized by microbial organisms for respiration. Carbon acts as the electron donor in these reactions, which may result in the loss of plant biomass. It is the loss of biomass, particularly the belowground biomass that has been implicated in degradation of coastal marshes. For instance, Deegan et al. (2012) found that nutrients added to a New England salt marsh increased above-ground leaf biomass, decreased the below-ground biomass of bank-stabilizing roots, and increased the microbial decomposition of organic matter, all of which resulted in creek-bank collapse and a subsequent conversion of those unvegetated

areas to mud. Wigand et al. (2014) examined the historical inputs of nutrients in wastewater loads into marshes in the urban Jamaica Bay Estuary, New York. They found that the Black Bank site exhibited lower abundance and biomass of roots and rhizomes, larger diameter rhizomes, a lower percentage of soil organic matter as well as higher carbon dioxide emission rates, greater peat particle density compared to the stable JoCo Marsh. In addition, Wigand et al. (2014) suggested that the Black Bank site had higher decomposition rates, increased peat decomposition, and highly waterlogged peat than the JoCo Marsh. Thus, anthropogenic inputs may reduce the ability of coastal wetlands to maintain soil elevation and keep pace with relative sea level rise. Pesticides and herbicide loads, which have been utilized to increase crop yields, have accompanied the nutrient loads associated with nonpoint source pollution runoff and may reduce wetland health.

The herbicide atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is used for pre-emergence and post-emergent control of broadleaf plants and grasses in agricultural and forestry operations (Ghosh and Philip 2006). Atrazine binds with a protein complex in Photosystem II in plant chloroplasts and inhibits the transfer of electrons, which in turn, disrupts the formation and release of oxygen (USEPA 2016). Atrazine may also undergo transformation in the soil, soil porewater, and water column into its primary metabolites deethylatrazine (DEA), deisopropylatrazine (DIA), and hydroxyatrazine (HA) (Clay and Koskinen 1990a, Clay and Koskinen 1990b, Seybold and Mersie 1996, Mersie et al. 1998). These metabolites may be further transformed along a degradation pathway to form cyanuric acid and then biuret by cleavage of the ring structure via hydrolysis (Kruger et al. 1993a, Kruger et al. 1993b). The end products of atrazine degradation are carbon dioxide and ammonia;

consequently, atrazine may be a potential source of additional nitrogen input to wetland macrophytes via the process of nitrification, which oxidizes ammonia and converts it to nitrate.

The effects of atrazine on agricultural crops are well known, but there is a lack of consensus about how atrazine affects wetland plants. For example, Bouldin et al. (2006) reported decreased root growth of *Juncus effusus* plants exposed to atrazine in a hydroponic solution despite any indications of observable stress. However, Lytle and Lytle (1998) found that *Spartina alterniflora* was highly tolerant to atrazine doses as high as 3.1 mg L^{-1}, whereas the growth of *Juncus roemerianus* was significantly inhibited at 3.8 mg L^{-1} (Lytle and Lytle 2005).The results of these and nutrient enrichment studies (Valiela et al. 1976, Darby and Turner 2008b, Bodker et al. 2015) indicate that concerns about the effects of atrazine and nutrient loads on the health of the belowground biomass of wetland plants is warranted. However, the interactive effects of nutrient loading and atrazine exposure on wetland plants have not been explored.

An interactive effect of nutrients and atrazine on wetland plants may occur because nitrogen and phosphorus are often transported with atrazine molecules from their primary places of origin, which are usually agricultural areas that apply both nutrients and herbicides. Studies have shown that excess nutrient loads can degrade the belowground biomass of wetland plants (Darby and Turner 2008a, Darby and Turner 2008b, Wigand et al. 2009, Deegan et al. 2012, Bodker et al. 2015), which may result in reduced soil strength (Turner 2011). Other researchers have demonstrated that atrazine can negatively affect the growth of wetland plants (Lytle and Lytle 2005, Bouldin et al. 2006). In addition, the combination of atrazine treatments with nutrient addition may produce negative effects on the biomechanical properties of *Poaceae* species. For example, Sharpe et al. (1989) recorded a 48% reduction in the tensile root strength of *Cynodon*

dactylon (Bermudagrass) sod 8 weeks after application (WAT) after the sod plots were subjected to monthly nitrogen (not specified) treatments of 0.5 kg /100 m^2 and two 2.2 kg ha^{-1} atrazine treatments that were administered 9 days apart by a three-wheel, CO_2-pressurized sprayer. Turner and Dickens (1987) and Turner et al. (1990) also recorded reductions in the tensile strength of *Eremochloa ophiuroides* (Centepedegrass) sods exposed to atrazine in concert with applications of nitrogen as NH_4N0_3. Eastin and Davis (1967) investigated the effects of atrazine on nitrogen metabolism in corn, soybean, and cotton in two soil culture experiments and one nutrient culture experiment. The nitrogen fraction in the form of 14 µg mL^{-1} of NH_4-N and 203 µg mL^{-1} NO_3-N within a nutrient solution (Hoagland and Arnon 1950) was added with atrazine concentrations with ranges of 0, 4 and 8 ppm for corn; 0, 0.1, and 0.25 ppm for cotton; and 0, 0.025, and 0.05 ppm for soybean. In all three experiments, they found that whenever the percent nitrogen was increased by the atrazine treatment, then this increase was coupled with a decrease in plant dry weight. In addition, Eastin and Davis (1967) concluded that atrazine increased the nitrogen percentage within the plants by a reduction in growth rather than an increase in nitrogen uptake, but there were no significant differences in the responses of the shoots or roots. However, they posited that this decrease in growth may have been the result of inhibition of photosynthesis by atrazine. The results of these studies suggest that there may be interactive effects of atrazine and nutrient loading on the tensile strength of wetland plants that could accelerate coastal land loss in areas such as the US Gulf Coast and exacerbate the effects of relative sea level rise.

Tensile strength is the resistance of material in tension to an external load (Niklas 1992, Niklas and Spatz 2012). In organic wetland soils, tensile root strength may be a measure of the resistance of roots and rhizomes to pulling forces that can uproot plants. Wetland macrophytes

may be subjected to tensile loads exerted by wind, waves, gravity, and grazing herbivores. The tensile strength of individual roots may be affected by intrinsic factors such as tissue composition, cell wall construction, species-specific anatomical attributes, root turgor pressure, osmotic potential, and plant adaptations to environmental conditions (Niklas 1992, Niklas and Spatz 2012). Therefore, chemical compounds that cause anatomical, physiological, or metabolic changes in plants may have the potential to affect the tensile strength of plant structures. *Spartina patens* (Ait.) Muhl., is a dominant emergent macrophyte of coastal wetland plant communities in the Atlantic and Gulf coasts of the United States, and it occupies 96% of Louisiana's brackish and intermediate marshes (Chabreck 1972). This species is exposed to atrazine and high nutrient loads via flow from the Mississippi River after agricultural harvesting operations in the Midwest and the Mississippi River Delta.

The objective of this study was to determine whether atrazine and different combinations of nutrient addition alters the tensile root strength of *S. patens*. Results are reported from two experiments examining the effects of interactions between atrazine and different levels of nutrients using the tensile root strength of *S. patens* as the main metric of response. The hypothesis is tested that atrazine and nutrient addition have synergistic effects on the belowground biomass of *S. patens* that reduce its tensile root strength.

MATERIALS AND METHODS

Atrazine-Nutrient Addition Interaction Experiment

Plants were grown under natural light conditions. This experiment was conducted in the Louisiana State University greenhouses at Baton Rouge, Louisiana. The factorial experimental design consisted of 6 nutrient and 3 atrazine levels as the main effects, with 4 replicates per level (6x3x4). *Spartina patens* plugs from Tampa Bay estuary were purchased from Green Seasons

Nursery (Tampa, FL). Each plug consisted of 7–12 stems growing from a 3.0 x 3.0 x 6.6 cm root mass. These plants did not have a pre-experiment exposure to atrazine. The samples were transplanted to 3.78-liter (1 gallon) glass jars filled with 3.0 L of a mixture of 65% sphagnum peat (Premier Sphagnum Peat Moss; 100% Canadian peat moss, no added fertilizer or nutrients), a 30% clay/silt mixture, and 5% sand. The sand, silt, and clay components were obtained by the LSU greenhouse staff from soil in the Sterlington soil series (coarse-silty, mixed thermic Typic Hapludalfs) located in the Mississippi River floodplain in West Baton Rouge Parish. The soil texture of clay/silt components was estimated by texture-by-feel field technique and determined to be sandy clay loam (Brady and Weil 2002).

The nitrogen and phosphorus nutrient treatments consisted of granular reagent grade calcium nitrate tetrahydrate [$Ca(NO_3)_2 \bullet 4H_2O$] and granular laboratory grade potassium phosphate [K_3PO_4] (Fisher Scientific; Nazareth, PA). Nutrient treatments, which were added bi-monthly in a 1-L deionized water solution, were as follows: High Nitrogen (HN, 5.0 mg L^{-1}), Low Nitrogen (LN, 1.75 mg L^{-1}), High Phosphorus (HP, 0.30 mg L^{-1}), Low Phosphorus (LP, 0.10 mg L^{-1}), High Nitrogen x Low Phosphorus (Np), and Low Nitrogen x High Phosphorus (nP). A 25 ppm atrazine stock solution was formed by placing Pestanal® Sigma-ALDRICH atrazine in deionized water (Starr et al. 2017). Because atrazine has a moderate solubility in water (30 ppm at 20 °C), the solution was placed on a hot plate with a magnetic stirrer, heated at 23 °C, and mixed with magnetic stirring rods for a 24 hour period before the experiment to ensure the atrazine was fully dissolved (Starr et al. 2017). The volume of atrazine required for each experiment treatment (V_2) was calculated by the equation $C_1V_1 = C_2V_2$, where C_1 and C_2 are the initial and final concentrations, respectively; and V_1 and V_2 are the initial and final volumes, respectively. Atrazine treatments, which were also added bi-monthly in a 1-L deionized water

solution, were: High (3.0 micrograms per liter [µg L^{-1}]), Medium (1.5 µg L^{-1}), and Low (0.5 µg L^{-1}). The transplants were acclimated for 8 weeks to adjust to greenhouse conditions. During the experiment, glass pots were rotated monthly on a reverse-orientation basis (e.g. south to north, west to east) to reduce the variation in environmental conditions. The water levels between treatments were maintained 1.75 cm above the soil surface to ensure saturated soil conditions. Soil temperature, pH, and redox potential were measured before the addition of nutrient and atrazine treatments. Soil temperature was measured by inserting a soil probe thermometer into each unit and recording the result to the nearest 0.1° C. The pH of the soil pore water was obtained by withdrawing a 175-mL sample of soil pore water with a Lisle vacuum pump (Lisle Corporation, Clarinda, IA) and dispensing the water into a 250-mL amber glass bottle. The soil pore water pH was measured by a Hach HQ 40d multi-parameter meter (Hach Industries Loveland, CO). The redox potential was measured with 45 cm-long standard platinum probes following the procedures of Reddy and Delaune (2008) and a Corning calomel reference probe (Corning, Inc. Corning, NY) that were connected to a Fluke 73 Multimeter (John Fluke Manufacturing, Everett WA). A correction of +244 mV was added to redox measurements to compensate for the difference in redox potential between the calomel probe and standard hydrogen reference electrode (Reddy and Delaune 2008). The experiment was conducted for a total of 212 days from 1 December 2015 until 30 June 2016.

Disturbed Controls Experiment

A disturbed control experiment was conducted to monitor the impact of the main effects on the plant samples. The experimental design consisted of eight replicates of each of the six nutrient treatments for the atrazine-nutrient interaction experiment, plus 8 control replicates. The atrazine disturbed control experiment also consisted of eight replicates of each of the three

atrazine treatments for the atrazine-nutrient interaction experiment, plus 8 control replicates. The plant samples, soil components, environmental conditions, and experimental set-up were exactly the same as the atrazine-nutrient interaction experiment. The experiment was conducted for a total of 60 days from 1 December 2015 until 30 January 2016.

Tensile Strength Testing

Tensile strength testing was conducted on live roots in only one of the five diameter size classes utilized by Hollis and Turner (2018). The Small size class (0.5–1.0 mm) was selected for testing because of the high numbers of roots within this diameter range and the increased probability of conducting successful tensile strength tests. Personal observation noted that a mean of six tests must be conducted for every successful tensile strength test. A successful test consisted of root samples that failed between the supports of the test stand, whereas roots that failed at the supports were considered unsuccessful tests and the data were invalid. Live roots and rhizomes were differentiated from dead roots by their white, turgid, and translucent appearance, whereas dead roots were dark and flaccid (Darby and Turner 2008b). However, many live roots were stained by soil deposits; they were separated from dead roots by the presence of turgor, bifurcations of fine roots, and their ability to float. Three individual root metrics were measured: mass, length, and diameter; while cross-sectional area and volume were calculated from these metrics. Root length was measured to the nearest 0.1 mm with a Scale Master© Classic digital planimeter (Calculated Industries, Carson, Nevada USA). The mean root diameter was measured to the nearest 0.1 mm with a Starrett digital IP67 micrometer. Measurements were taken at both ends and at the middle of each root and then averaged. Cross-sectional area (mm^2) and volume (mm^3) were calculated from length and diameter measurements after tensile strength testing was performed. Root samples were weighed to estimate individual

mass to the nearest 0.1 (mg). A Mecmesin MultiTest –d motorized stand (Mecmesin Limited; Sinfold, West Sussex, United Kingdom) was used to test tensile root strength in Newtons (N). Individual roots were secured to two support clamps aligned perpendicular to the base of the test stand. The contact surfaces of the clamps provided an area of 1.25 x 2.50 cm and were lined with fine sandpaper to reduce or eliminate slippage. In addition, the support clamps were attached to a Mecmesin Basic Force Gage load meter, which was capable of measuring 1000 N of force with a precision of 0.1 N. The test stand was activated and the top support was pulled upward by a vertical hydraulic piston until the root exhibited structural failure. The load that induced failure at that point, or breaking force, was recorded as tensile strength.

Tissue Sample Testing

Samples of live leaf and root tissue for each of the interaction experiment units and the control were collected after tensile strength testing at the end of the experiment and sent to the LSU Soil Testing and Plant Analysis Laboratory for determination of the carbon, nitrogen, and phosphorus tissue content. These were used to calculate carbon-nitrogen (C:N) and nitrogen-phosphorus ratios (mM g^{-1}). Samples of live leaf and root tissue from each of the interaction experiment units and from the control, as well as soil and soil porewater samples were analyzed for atrazine concentration by the LSU Department of Agricultural Chemistry. The detection limit for leaf and root samples was 25 µg L^{-1}; however, the detection limit for porewater samples was 0.1 µg L^{-1}.

Statistical Analyses

I conducted a one-way analysis of variance (ANOVA) in JMP v. 13 statistical software (SAS Cary, NC) to test for significant differences between the nutrient and atrazine main effects and their respective controls in the disturbed controls experiment. Tests to determine any differences in the mean tensile strength of roots by soil texture and atrazine treatment in the

atrazine-nutrient interaction experiment were made using ANOVA in JMP v. 13. The interactive effects were determined by segregating the tensile root strength data of the levels of one main effect into subsets and then conducting one-way ANOVA of tensile root strength using each level of the other main effect. For instance, the tensile root strength data were divided by the three levels of the atrazine main effect into High (3.0 µg L^{-1}), Medium (1.5 µg L^{-1}), and Low (0.5 µg L^{-1}) subsets and then one-way ANOVAs of tensile root strength were conducted for each of the six levels of the nutrient addition main effect (e.g. Tensile strength x High Nitrogen using the High atrazine data subset). I used a Tukey-Kramer Honest Significant Difference (HSD) test in both experiments, tests to determine if there were any significant differences between the tensile root strength means. The data are reported as the mean ± 1 standard error of the mean unless otherwise noted. Homoscedasticity and normality of residuals were determined with Brown-Forsythe and Shapiro-Wilk tests, respectively. The data that did not meet the assumptions of an ANOVA were tested with a Welch's ANOVA and the differences between the tensile strength means were determined using a Steel-Dwass nonparametric multiple comparison test. Interactive effects of treatment combinations were determined by using a Kolmogorov-Smirnov goodness-of-fit test to compare the data distribution of the combination with that of the strongest main effect of the treatment combination. A Student's *t*-test was used to test for statistical significance among the soil temperature, redox potential, and pH parameters. The differences among the nutrient and the carbon:nitrogen:phosphorus (C:N:P) ratios were tested with a one-way ANOVA. All statistical tests were performed at a significance level of $p < 0.05$.

RESULTS

Disturbed Controls Experiment

A one-way ANOVA detected no significant difference in tensile root strength in either the atrazine treatments or Control (Fig 4.1a, $F = 1.002$, $p = 0.3934$) or the nutrient treatments and Control (Fig 4.2a, $F = 1.076$, $p = 0.3809$). In addition, there was no significant difference in tensile root strength among the atrazine or nutrient treatments. The grand means of the tensile root strength between the atrazine and nutrient treatments and Control were 4.6 ± 0.30 N and 4.4 ± 0.39 N, respectively. The pH ranges for the High, Medium, and Low atrazine treatments were 6.9 to 7.4 (7.2 ± 0.02), 6.9 to 7.1 (7.0 ± 0.02), and 6.8 to 7.2 (7.0 ± 0.02), respectively (Data not shown). The pH of the Control ranged from 6.8 to 7.3 (7.0 ± 0.03). The mean air temperature within the greenhouse during the experiment was 27.6 °C. The soil temperature ranges for the High, Medium, and Low atrazine treatments were 27.0–31.6 °C (29.3 ± 0.5 °C), 26.8–32.1 °C (29.1 ± 0.5 °C), and 26.9–33.1 °C (30.1 ± 0.5 °C), respectively. The redox potential ranges for the High, Medium, and Low atrazine treatments were –21.0 to +19.8 mV (–1.3 ± 0.9 mV), –15.9 to +4.9 mV (–5.3 ± 1.5 mV), and –50.2 to +10.7 mV (–20.5 ± 1.2 mV), respectively.

Atrazine-Nutrient Addition Interaction Experiment

A one-way Welch's ANOVA detected significant differences in the tensile root strength between all atrazine treatments and Control (Fig. 4.1b, $F = 16.4$, $p < 0.0001$); however, there were no significant differences among the tensile root strength of the atrazine treatments, and the grand tensile root strength mean was 2.48 ± 0.23 N. A one-way Welch's ANOVA revealed a significant difference in the tensile root strength between all nutrient treatments and Control (Fig. 4.2b, $F = 8.50$, $p < 0.0001$); however, there were no significant differences in tensile root strength among the six nutrient treatments and the grand mean was 2.29 ± 0.19 N.

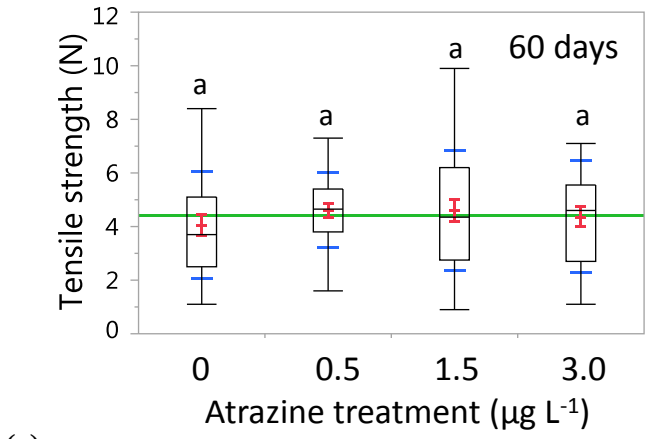
A one-way Welch's ANOVA of tensile root strength in the High Nitrogen (HN) and Low Nitrogen subsets revealed significant differences in tensile root strength between all atrazine treatments and Control (Fig. 4.3a, $F = 16.3$, $p < 0.0001$; Fig. 4.3b, $F = 23.1$, $p < 0.0001$, respectively); however, there were no significant differences among the tensile root strength of the atrazine treatments for either subset. The grand means of tensile root strength for the HN and LN subsets were 2.60 ± 0.22 and 2.36 ± 0.21 N, respectively.

A one-way Welch's ANOVA of tensile root strength in the High Phosphorus (HP) and Low Phosphorus (LP) subsets revealed significant differences in tensile root strength between all atrazine treatments and Control (Fig. 4.3c, $F = 27.0$, $p < 0.0001$; Fig. 4.3d, $F = 22.2$, $p \leq 0.0002$, respectively). There were significant differences between the tensile root strength of the High and Medium atrazine treatments for the HP subset ($p = 0.049$) as well as between the High and Low atrazine treatments for the LP subset ($p = 0.0026$). The tensile root strength grand means for the HP and LP subsets were 2.31 ± 0.22 and 2.70 ± 0.23 N, respectively.

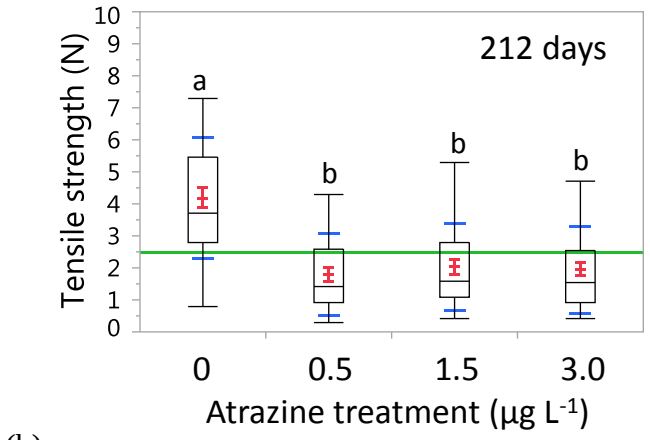
Significant differences in tensile root strength between all atrazine treatments and Control were revealed by a one-way Welch's ANOVA of tensile root strength in the nitrogen-phosphorus combination subsets (Np and nP) (Fig. 4.3e, $F = 20.8$, $p < 0.0001$; Fig. 4.3f, $F = 14.1$, $p \leq 0.0001$, respectively). However, there were no significant differences among the tensile root strength atrazine treatments for either nutrient subset ($p > 0.05$). The tensile root strength grand means for the Np and nP subsets were 2.69 ± 0.27 and 2.40 ± 0.21 N, respectively.

A one-way Welch's ANOVA of the High atrazine treatment subset found significant differences in tensile root strength between all nutrient treatments and Control (Fig. 4.4a, $F = 15.7$, $p < 0.0001$); and there were significant differences in tensile root strength among the LP and the LN, nP, and Np nutrient treatments ($p > 0.03$). The tensile root strength grand mean for

the High subset was 2.28 ± 0.21 N. A Kolmogorov-Smirnov goodness-of-fit test revealed interactive effects in the HPxH and LPxH subsets (Prob |D| < 0.05, Table 4.1).

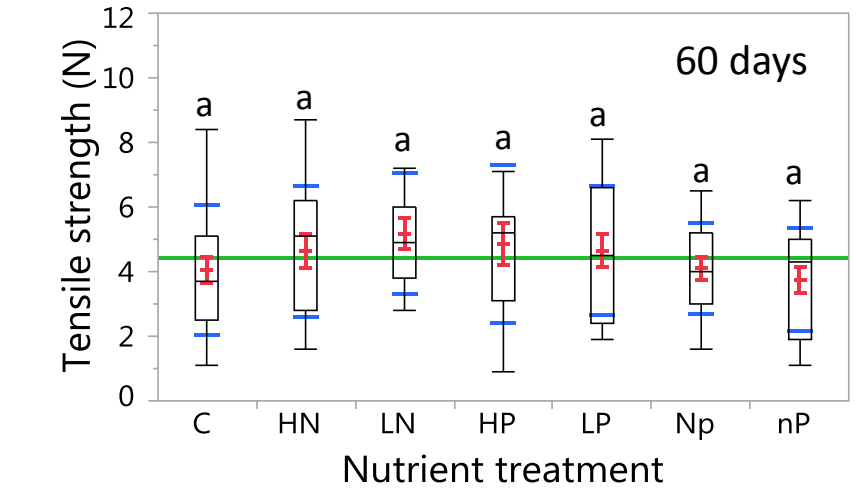


(a)

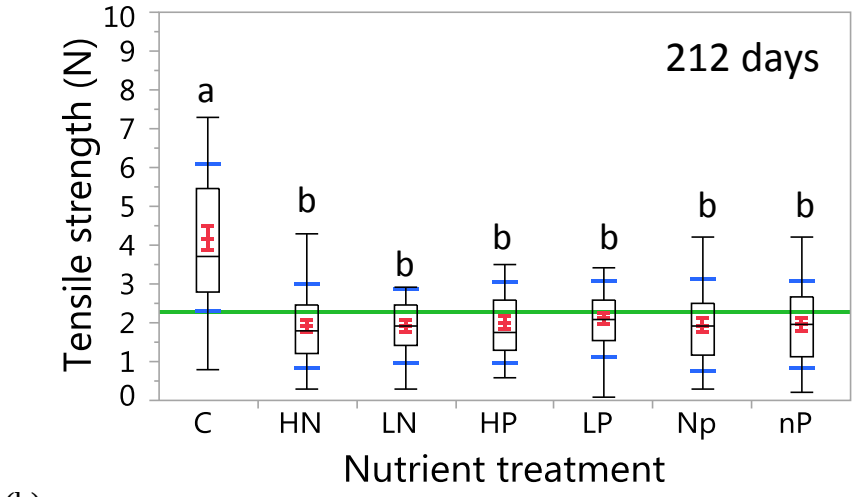


(b)

Fig. 4.1 Box-and-whisker plots of (a) a one-way ANOVA of tensile root strength with atrazine as the main effect for the atrazine control greenhouse experiment (b) one-way Welch's ANOVA of tensile root strength with atrazine as the main effect for the atrazine-nutrient interaction greenhouse experiment. Tensile root strength in the control (0 µg L^{-1}) was significantly higher than in low (0.5 µg L^{-1}), medium (1.5 µg L^{-1}), and high (3.0 µg L^{-1}) atrazine treatments (Table 4.2, F= 16.4, $p < 0.0001$). There was no significant differences between control and atrazine treatments or among atrazine treatments (p = 0.3934). The box plot whiskers represent the sample range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line is the grand mean for all groups. Box plots with different letters denote significant differences between treatments



(a)



(b)

Fig. 4.2 Box-and-whisker plots of (a) a one-way ANOVA of tensile root strength with nutrient addition as the main effect for the nutrient control greenhouse experiment (b) one-way Welch's ANOVA of tensile root strength with nutrient addition as the main effect for the atrazine-nutrient interaction greenhouse experiment. The tensile root strength in the control was significantly higher than in the nutrient treatments (F= 12.6, $p < 0.0001$). There was no significant difference between control and nutrient treatments ($p = 0.3809$) or among nutrient treatments ($p > 0.05$) for the 60-day control experiment. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

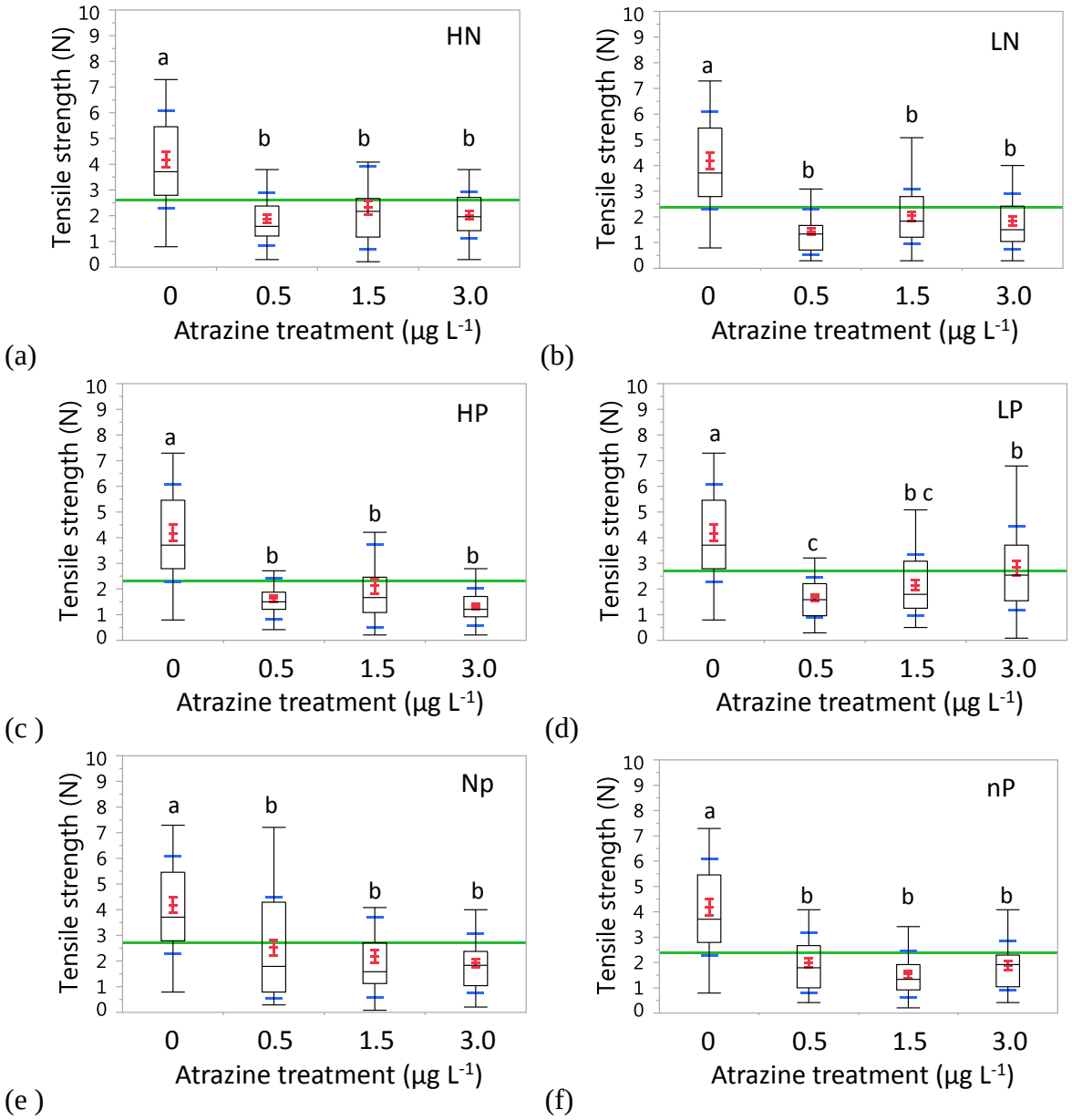


Fig. 4.3 Box-and-whisker plots of one-way Welch's ANOVA of tensile root strength with atrazine as the main effect for (a) the High Nitrogen (HN) (b) the Low Nitrogen (LN) (c) High Phosphorus (HP) (d) Low Phosphorus (LP) (e) High Nitrogen-Low Phosphorus (Np) (f) Low Nitrogen-High Phosphorus (nP) treatment subsets to test for interactive effects between nutrient and atrazine treatments. There were significant differences between control and atrazine treatments for all subsets (Table 4.1, $p < 0.0001$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

Table 4.1 Summary statistics of the tensile root strength response variable for the atrazine treatment and nutrient addition main effects and main effect subsets (in parentheses) used to test for interactive effects (Treatment means in bold denote significant interactions). Statistical significance is indicated by *p*-values < 0.05

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Nutrient	280	n/a	n/a	n/a	1.98	2.29	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.19	1.90	n/a
High Nitrogen (HN)	40	6.2	0.3	1.92	n/a	n/a	0.19	1.08	< 0.0001
Low Nitrogen (LN)	40	5.1	0.3	1.91	n/a	n/a	0.19	0.96	< 0.0001
High Phosphorus (HP)	40	6.0	0.6	2.01	n/a	n/a	0.19	1.02	< 0.0001
Low Phosphorus (LP)	40	5.7	0.1	2.11	n/a	n/a	0.19	0.98	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	6.4	0.3	1.94	n/a	n/a	0.19	1.18	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	5.7	0.2	1.96	n/a	n/a	0.19	1.11	< 0.0001
Nutrient (Low)	280	n/a	n/a	n/a	1.85	2.18	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.21	1.90	n/a
High Nitrogen (HN)	40	4.8	0.3	1.87	n/a	n/a	0.21	1.02	< 0.0001
Low Nitrogen (LN)	40	4.7	0.3	1.41	n/a	n/a	0.21	0.89	< 0.0001
High Phosphorus (HP)	40	4.7	0.4	1.64	n/a	n/a	0.21	0.80	< 0.0001
Low Phosphorus (LP)	40	3.2	0.3	1.66	n/a	n/a	0.21	0.80	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	7.2	0.3	2.52	n/a	n/a	0.21	1.99	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	6.2	0.4	1.98	n/a	n/a	0.21	1.18	< 0.0001

(Table 4.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Nutrient (Medium)	280	n/a	n/a	n/a	2.05	2.35	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.23	1.90	n/a
High Nitrogen (HN)	40	4.8	0.3	2.31	n/a	n/a	0.23	1.60	< 0.0001
Low Nitrogen (LN)	40	4.7	0.3	2.02	n/a	n/a	0.23	1.07	< 0.0001
High Phosphorus (HP)	40	4.7	0.4	2.11	n/a	n/a	0.23	1.62	< 0.0001
Low Phosphorus (LP)	40	3.2	0.3	2.15	n/a	n/a	0.23	1.20	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	7.2	0.3	2.15	n/a	n/a	0.23	1.57	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	6.2	0.4	1.54	n/a	n/a	0.23	0.92	< 0.0001
Nutrient (High)	280	n/a	n/a	n/a	1.97	2.28	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.20	1.90	n/a
High Nitrogen (HN)	40	3.8	0.3	2.02	n/a	n/a	0.20	0.90	< 0.0001
Low Nitrogen (LN)	40	4.7	0.3	1.84	n/a	n/a	0.20	1.10	< 0.0001
High Phosphorus (HP)	40	3.5	0.2	1.32	n/a	n/a	0.20	0.72	< 0.0001
Low Phosphorus (LP)	40	6.8	0.1	2.82	n/a	n/a	0.20	1.65	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	5.2	0.2	1.92	n/a	n/a	0.20	1.17	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	4.8	0.4	1.88	n/a	n/a	0.20	0.95	< 0.0001

(Table 4.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Atrazine	160	n/a	n/a	n/a	1.91	2.48	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.23	1.90	n/a
Low	40	6.8	0.3	1.78	n/a	n/a	0.23	1.28	< 0.0001
Medium	40	6.5	0.4	2.02	n/a	n/a	0.23	1.34	< 0.0001
High	40	6.6	0.4	1.94	n/a	n/a	0.23	1.35	< 0.0001
Atrazine (HN)	160	n/a	n/a	n/a	2.06	2.60	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.22	1.90	n/a
Low	40	4.8	0.3	1.87	n/a	n/a	0.22	1.02	< 0.0001
Medium	40	6.6	0.2	2.31	n/a	n/a	0.22	1.60	< 0.0001
High	40	3.8	0.3	2.02	n/a	n/a	0.22	0.90	< 0.0001
Atrazine (LN)	160	n/a	n/a	n/a	1.76	2.36	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.21	1.90	n/a
Low	40	4.7	0.3	1.41	n/a	n/a	0.21	0.89	< 0.0001
Medium	40	5.1	0.3	2.02	n/a	n/a	0.21	1.07	< 0.0001
High	40	4.7	0.3	1.84	n/a	n/a	0.21	1.10	< 0.0001
Atrazine (HP)	160	n/a	n/a	n/a	1.69	2.31	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.22	1.90	n/a
Low	40	4.7	0.4	1.64	n/a	n/a	0.22	0.80	< 0.0001
Medium	40	6.8	0.2	2.11	n/a	n/a	0.22	1.62	< 0.0001
High	40	3.5	0.2	**1.32**	n/a	n/a	0.22	0.72	< 0.0001

(Table 4.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Atrazine (LP)	160	n/a	n/a	n/a	2.21	2.70	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.23	1.90	n/a
Low	40	3.2	0.3	1.66	n/a	n/a	0.23	0.80	< 0.0001
Medium	40	5.1	0.5	2.15	n/a	n/a	0.23	1.20	< 0.0001
High	40	6.8	0.1	**2.82**	n/a	n/a	0.23	1.65	0.0002
Atrazine (Np)	160	n/a	n/a	n/a	2.24	2.69	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.27	1.90	n/a
Low	40	7.2	0.3	2.52	n/a	n/a	0.27	1.99	0.0001
Medium	40	7.4	0.1	2.16	n/a	n/a	0.27	1.57	< 0.0001
High	40	5.2	0.2	1.92	n/a	n/a	0.27	1.17	< 0.0001
Atrazine (nP)	160	n/a	n/a	n/a	1.76	2.40	n/a	n/a	< 0.0001
Control	40	9.9	0.8	4.19	n/a	n/a	0.21	1.90	n/a
Low	40	6.2	0.4	1.98	n/a	n/a	0.21	1.17	< 0.0001
Medium	40	4.4	0.2	1.54	n/a	n/a	0.21	0.92	< 0.0001
High	40	4.8	0.4	1.88	n/a	n/a	0.21	0.95	< 0.0001

In the Medium atrazine subset, there were significant differences in tensile root strength between all nutrient treatments and Control (Fig. 4.4b, $F = 10.4$, $p < 0.0001$). There were no significant differences in tensile root strength among the nutrient treatments ($p > 0.05$).

Table 4.2 Summary of one-way Welch's ANOVA tests of the tensile root strength response variable for the atrazine treatment and nutrient addition main effects and main effect subsets (in parentheses) used to test for interactive effects. Statistical significance is indicated by $p < 0.05$

Source	[1]DFNum	[2]DFDen	F Ratio	*p*-value
Nutrient	6	121.0	8.50	< 0.0001
Nutrient (High)	6	108.2	15.9	< 0.0001
Nutrient (Medium)	6	107.3	16.4	< 0.0001
Nutrient (Low)	6	107.7	17.9	< 0.0001
Atrazine	3	86.0	16.4	< 0.0001
Atrazine (HN)	3	83.8	16.3	< 0.0001
Atrazine (LN)	3	84.9	23.1	< 0.0001
Atrazine (HP)	3	82.3	27.0	< 0.0001
Atrazine (LP)	3	82.1	22.2	< 0.0001
Atrazine (Np)	3	84.7	14.2	< 0.0001
Atrazine (nP)	3	84.6	20.8	< 0.0001

[1]Degrees of Freedom -Numerator; [2]Degrees of Freedom - Denominator

The tensile root strength grand mean for the Medium subset was 2.35 ± 0.21 N. A one-way Welch's ANOVA of the tensile root strength in the Low atrazine treatment subset found significant differences between all nutrient treatments and Control (Fig. 4.4c, $F = 12.95$, $p < 0.0001$). In addition, there were significant differences in tensile root strength among the Np and the LN ($p = 0.0036$) and HP ($p = 0.0429$) nutrient treatments. The tensile root strength grand mean for the Low subset was 2.18 ± 0.21 N.

Soil Parameters

The mean soil temperature in the experimental units ranged from 26.1 to 26.6°C (Table 4.3; Appendix B, Fig B1) with an overall mean of 26.3 ± 0.41 °C, and less than 1°C variation

between the mean temperatures for each soil texture. A Student's t-test revealed no significant difference between the soil temperatures among the three atrazine treatments or control (p>0.05).

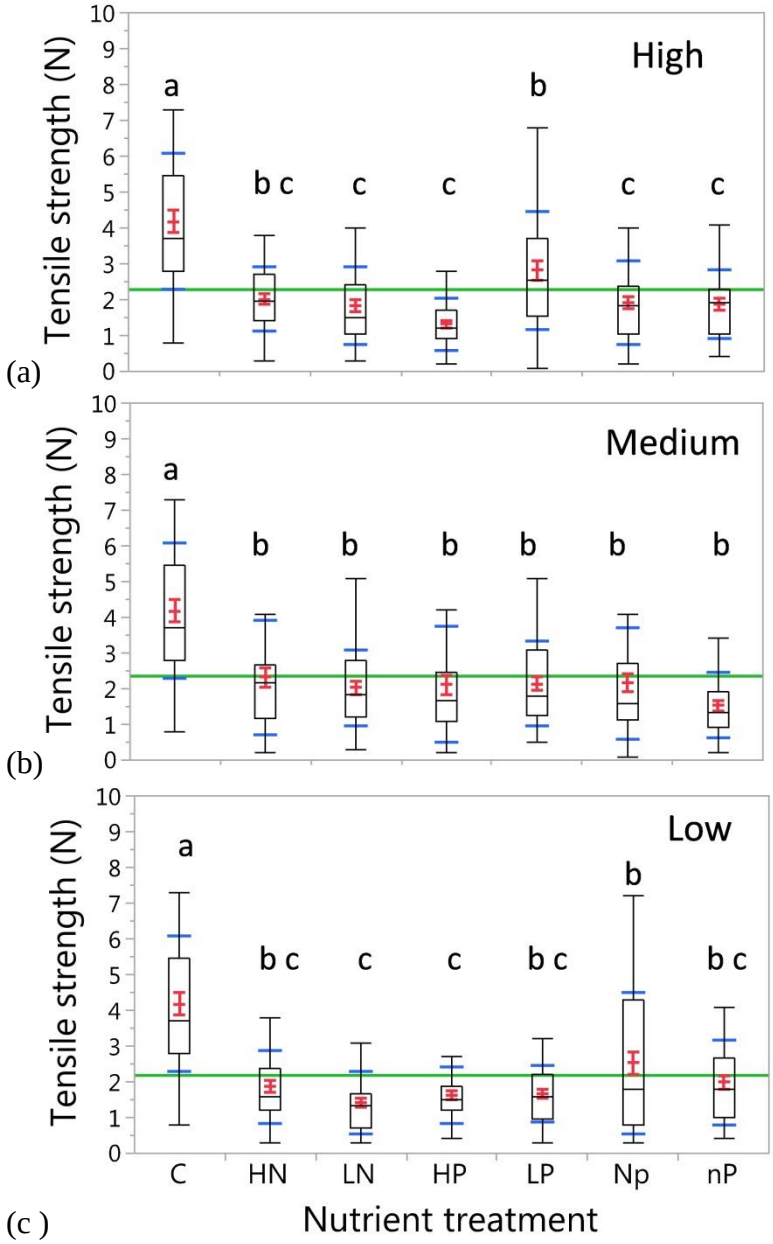


Fig. 4.4 Box-and-whisker plots of one-way Welch's ANOVA of tensile root strength with nutrient addition as the main effect for (a) the High Atrazine (b) the Medium Atrazine (c) Low Atrazine treatment subsets to test for interactive effects between nutrient and atrazine treatments. There were significant differences between control and nutrient treatments for all subsets (Table 4.1, $p < 0.0001$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

The pH of the experimental units was neutral to alkaline throughout the experiment and the mean pH was 7.1 in all three atrazine treatments (Table 4.3; Appendix B, Fig. B2). As a result, a Student's t-test found no significant differences between the three and the Control.

The redox potential fluctuated frequently between the experimental units throughout the duration of the experiment. There was less than 6 mV of variation between the redox potential means of the experimental units and control (Table 4.3; Appendix B, Fig B3). Consequently, a Student's t-test revealed no significant differences in the soil redox potential among the three atrazine treatments and Control ($p > 0.05$).

Plant Tissue Nutrient Content

The carbon content of *S. patens* above- and belowground tissue varied between the nutrient treatments. With the exception of the High Nitrogen (HN) treatment, a greater concentration of carbon was detected in the roots than in the aboveground (Stem) tissue (Table 4.4). A one-way ANOVA revealed that the carbon content in the stems for the HP, LP, and nP units were significantly higher than the Control ($F = 12.9$, $p < 0.0001$).
However, the LN and HN units were not significantly different than the Control. The C:N ratio in the roots was less than 100; however, in the stems, the C:N ratio ranged from 85 in the nP units to 100.9 in LN units. Similarly, there were greater concentrations of nitrogen and phosphorus in the roots than in the stems. In the roots, the N:P ratios ranged from 7.2 in the HP units to 10.6 in the Control. In the stems, the N:P ratios ranged from 9.6 in the HN and LN units, respectively, to 11.3 in the Low Nitrogen-High Phosphorus (nP). A one-way ANOVA revealed that the nitrogen content in the roots for the LP and nP units were significantly higher than the Control; however, in the stems, the Np and nP units were significantly higher than Control ($F = 7.9$, $p < 0.0001$).

Table 4.3 Summary of soil parameters of a nutrient-atrazine interaction experiment delineated by atrazine treatment. Mean values with different letter subscripts are significantly different (p<0.05)

Parameter	Experimental Treatments			
	Low	**Medium**	**High**	**Control**
Soil Temperature (°C)				
Mean	26.6[a]	26.3[a]	26.3[a]	26.1[a]
Min	19.7	19.8	19.8	19.9
Max	31.4	31.2	31.3	31.0
Standard Error	0.45	0.40	0.41	0.38
pH				
Mean	7.1[a]	7.1[a]	7.1[a]	7.1[a]
Min	7.0	7.0	7.0	6.9
Max	7.4	7.3	7.3	7.6
Standard Error	0.02	0.01	0.01	0.03
Redox Potential (mV)				
Mean	–12.3[a]	–14.0[a]	–14.4[a]	–17.5[a]
Min	–26.6	–22.2	–23.3	–30.1
Max	–3.6	–7.3	–7.8	–4.8
Standard Error	0.8	0.6	0.6	1.1

Atrazine Testing

Neither atrazine nor any of its primary metabolites were detected in leaf, root, or solid soil samples from any of the Low, Medium, or High atrazine experimental units. The detection limit for these samples was 25 µg L^{-1}; however, the detection limit for water and soil porewater samples was 0.1 µg L^{-1}. Atrazine was detected in the soil porewater of the Low, Medium, and High atrazine units at a concentration of 0.0083 µg L^{-1}, 0.0095 µg L^{-1}, and 0.0435 µg L^{-1}, respectively. In addition, atrazine and DEA were detected in the deionized water controls at mean concentrations of 6.96 and 1.60 µg L^{-1}, respectively.

Table 4.4 Results of nutrient tissue content testing of live *S. patens* above- (stem) and belowground biomass (roots) for carbon, nitrogen, and phosphorus as well as carbon-nitrogen (C:N) and nitrogen-phosphorus (N:P) ratios. Mean values with different letter subscripts are significantly different ($p<0.05$). Comparisons of means were made within each nutrient between treatments and control as well as between roots and stems

Treatment	**Carbon (mmol/g)**		**Nitrogen (mmol/g)**		**Phosphorus (mmol/g)**		**C:N**		**N:P**	
	Roots	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*
HN	36323^{a}	35997^{ac}	468.8^{c}	376.1^{de}	48.3^{a}	39.1^{ac}	77.5	95.7	9.7	9.6
LN	36023^{a}	36261^{ac}	500.6^{bc}	359.3^{d}	59.4^{b}	37.5^{ac}	72.0	100.9	8.4	9.6
HP	36134^{a}	37033^{d}	473.3^{c}	387.3^{de}	65.3^{b}	39.1^{ac}	76.4	95.6	7.2	9.9
LP	36393^{a}	37164^{d}	553.1^{ab}	381.0^{de}	61.2^{b}	36.5^{ac}	65.8	97.5	9.0	10.4
Np	35573^{b}	36456^{cd}	524.8^{abc}	405.3^{ef}	61.9^{b}	37.3^{ac}	67.8	89.9	8.5	10.9
nP	34656^{b}	37069^{d}	580.0^{a}	431.8^{cf}	59.4^{b}	38.3^{ac}	59.8	85.8	9.8	11.3
Control	36237^{ac}	36546^{ac}	454.1^{c}	363.3^{d}	43.0^{a}	32.3^{a}	79.8	100.6	10.6	11.2

DISCUSSION

The tensile root strength of *S. patens* exhibited several responses to an atrazine-nutrient interaction experiment. These effects are discussed next in terms of soil parameters, atrazine exposure, nutrient addition, and the interactive effects.

Soil Parameters

The soil parameters were key indicators of the response of *S. patens* to atrazine exposure, nutrient addition, and the interactive effects of both treatments. The potential effects of atrazine and nutrients on tensile root strength are dependent upon their fate in the soil and their availability to the plant. In turn, soil conditions such as pH, temperature, soil texture, and redox potential have a direct effect on nutrient dynamics and plant atrazine assimilation. With both main effects, the first step in degradation of tensile root strength was dependent upon plant assimilation of the atrazine, nutrients, or both. Therefore, the attributes of the soil parameters were probably the primary factors that facilitated the adverse effects of the atrazine and nutrient treatments on the roots.

Soil temperature can affect both nutrient cycling and the fate of atrazine. For example, the rate of chemical reactions can increase with increasing temperature. The rates of chemical reactions double with every 10 °C increase in temperature. Consequently, respiration rates and nutrient cycling processes such as denitrification can increase and exact a carbon demand on the plant. Because carbon is used as an electron donor for these processes, the tensile root strength may be affected by the loss of structural material. The addition of alternate electron acceptors in the experimental units (e.g. nitrate in calcium nitrate tetrahydrate) provided the catalyst for use of plant tissue as a carbon donor. The higher temperatures may have increased the rate of carbon loss in the roots and decreased the tensile root strength.

The solubility of atrazine can be affected by temperature. As temperatures increase, then atrazine becomes more soluble because of conditions that are conducive to severing molecular bonds. As atrazine becomes more soluble, then its availability to plants increases. Therefore, the soil temperature in the experimental units may have facilitated atrazine uptake and a subsequent decline in tensile root strength. Atrazine (and its possible interaction with nutrient addition) is implicated because the Control units were subjected to the same temperatures as the experimental units, but the mean tensile root strength of the Control units was nearly 50% greater and they produced considerably more root biomass (Fig. 4.5, 4.6).

The pH of the soil may have affected tensile root strength because of its influence on atrazine availability and nutrient cycling dynamics. For example, the adsorption of atrazine onto organic and mineral soil colloids may be affected by pH. Ionized humic acids can adsorb protonated atrazine molecules by ionic bonding (Choudhry 1983), whereas less atrazine adsorption occurs under alkaline conditions (McGlamery and Slife 1966). The mean pH in all experimental units was maintained at or above 7.0 for the duration of the experiment. As a result, there may have been a higher probability of atrazine availability and lower rates of adsorption, if any adsorption occurred at all. However, the availability of adsorbed atrazine is a function of time and pH; the longer the herbicide molecules are bound to the substratum, the more time and energy will be required to extract them (Mandelbaum et al. 2008). The soil pH not only affects atrazine adsorption, but it may alter nutrient dynamics as well. For instance, acidic conditions can facilitate the precipitation of phosphorus from metal complexes with iron. The pH also affects the partitioning of a compound between the solute and solution (McGlamery and Slife 1966). The soil pH can affect nutrient cycling indirectly by directly affecting microbial communities, which are also sensitive to the redox potential of the soil.

The redox potential remained below zero throughout the experiment. The highly anaerobic conditions in the soil were conducive to iron and manganese reduction. No plants displayed signs that iron was acting as a phytotoxin. The radial oxygen loss (ROL) from the roots and a considerably oxidized rhizosphere were visibly apparent in the experimental units because of the bright reddish redoximorphic features in the soil. Atrazine adsorption and degradation are generally curtailed under anaerobic conditions, but they are rapid under aerobic conditions (McGlamery and Slife 1966). However, the oxidized rhizosphere may have provided pockets along the root channels where atrazine assimilation, degradation, and/or transformation were possible. Consequently, the ROL could have nullified the effect of the anaerobic conditions on atrazine adsorption, which would have allowed plant uptake and subsequent effects on tensile root strength. Likewise, the oxidize rhizosphere would have affected nutrient dynamics as well. For instance, iron oxidation and reduction can be reversible in a soil matrix with aerobic and anaerobic interfaces. Ferrous iron in the reduced anaerobic soil may be oxidized to ferric iron in the oxidized rhizosphere and vice versa. Consequently, the availability of phosphorus will be affected by the redox potential as ferric iron can form metal complexes with phosphorus that reduces its availability to plants (Reddy and Delaune 2008). However, the plants in this experiment had more P concentrated in roots than in the aboveground biomass, which suggests that P uptake was not severely curtailed. Phosphorus is a key nutrient for plant growth; therefore if the plants were able to obtain a sufficient supply of P, additional root growth may have been suspended. As a result, the belowground biomass would have diminished because of other nutrient cycling processes (such as denitrification) that use carbon as an electron donor in redox reactions. Therefore, the effects of suspended growth and tissue degradation from redox

reactions and the inhibition photosynthesis by atrazine could have manifested as reduced tensile root strength.

Atrazine Exposure

The tensile root strength of *S. patens* declined after exposure to all three levels of atrazine. Atrazine was detected in the soil porewater at concentrations less than 0.1 μg L^{-1} in all three treatment units. In addition, atrazine and deethylatrazine (DEA) were detected in the deionized water controls at concentrations above the range of the treatment levels (1.60 to 6.96 μg L^{-1} vs. 1.0 to 5.0 μg L^{-1}). However, the herbicide was not detected in the leaf, root, or soil samples (25 μg L^{-1} detection limit). These results suggest that atrazine did not undergo rapid photodegradation in the water column, and the lack of detection in the soil samples indicates that adsorption may not have been a major contributor to the fate of atrazine doses. On the other hand, the soil porewater results may be an indication of desorption of atrazine molecules that had been adsorbed by the organic-dominated soil. Furthermore, the absence of the primary metabolites in the soil and water column samples suggests that the atrazine doses were not present in these areas long enough to undergo transformation. Davis et al. (1965) reported that the uptake of atrazine in corn occurred in period of 12 to 100 hours, which suggests that given the duration of this experiment; it is highly likely that the herbicide was assimilated by *S. patens*.

Nutrient Addition

Nutrient cycling can be influenced by the effects of soil texture, soil temperature, pH and redox potential. For instance, soil texture is a major driver of soil saturation and field capacity conditions (both micropores and macropores are flooded) change the biogeochemistry of the soil. Anaerobiosis affects the fate of nitrogen species such as nitrate, which may be reduced by denitrification. The denitrification of nitrate in the experimental units would have required

carbon as an electron donor. Consequently, the tensile root strength may have declined because of a reduction in the structural integrity of roots as microbes utilized carbon to reduce the nitrate additions. The balance between nitrogen and phosphorus uptake may have also affected tensile root strength. The N:P ratios for all experimental units were <33, which is an indication that nitrogen was the limiting nutrient to growth. However, as shown in Table 4.4, the addition of phosphorus, even at the lowest dose (LP) resulted in an increase in nitrogen concentration. Furthermore, resource partitioning of nutrients between the above- and belowground biomass indicates that the bulk of the assimilated nitrogen was stored in the aboveground biomass, while the phosphorus concentrated in the belowground biomass. Also, phosphorus may accumulate in the plant tissue because there is no biogeochemical process such as denitrification to remove it from the system. With a surfeit of nutrients, the roots in the experimental units may have ceased to grow, which is consistent with the optimum foraging theory and marginal value theorem.

Main Effects-Soil Parameter Dynamics

Soil texture is the nexus in which the interactive effects of soil temperature, soil pH, and redox potential may influence nutrient dynamics and atrazine distribution. The mineral or organic components can produce fine or coarse texture, respectively, as well as micropores and macropores, respectively. The structure and texture of the soil can determine moisture content; in turn, the moisture content of the water directly affects soil biogeochemical factors such as pH and redox potential. Soil temperature can influence solute solubility and increase the rate of reactions. For example, based on the results of other studies, the soil temperature range of the experimental units were moderately conducive to atrazine adsorption. In addition, the pH range was alkaline, which was also reported to be favorable for atrazine adsorption. Therefore, the effects of these two soil parameters may have facilitated plant assimilation of atrazine. On the

other hand, the redox potential was antagonistic in regard to adsorption in that less atrazine is adsorbed under anaerobic conditions. However, the combined effects of temperature and pH may have overcome the effects anaerobic conditions. Nutrient cycling is also influenced by soil texture and the subsequent conditions created by the soil texture. Mineral soils with large clay and/or silt components may be poorly drained, which may induce hypoxic or anoxic conditions that reduce nutrient ions in the soil. Under anaerobic conditions, these ions act as alternate electron acceptors for redox reactions. The organic component of the soil contributes carbon to these reactions as an electron donor to facilitate metabolic processes and soil temperature can increase the rate of these reactions. Consequently, root biomass may diminish as these processes proceed and result in the loss of tensile root strength.

The combination of nutrient addition and atrazine exposure drastically altered the root architecture of the treated plants (Fig. 4.5, 4.6). The effect of the atrazine-nutrient treatment combination was similar, no matter the combination (e.g. High atrazine x Low Phosphorus or Low atrazine x High nitrogen). During the first 50-day atrazine experiment (Chapter 3), atrazine doses were added to *S. patens* samples on a weekly basis for 7 weeks. The mean tensile root strength for the units in this experiment was 4.61 ± 0.43 N; whereas the mean tensile root strength of the nutrient treatment units in the High atrazine subset in the interaction experiment was 1.96 ± 0.20 N. It is important to note that the main difference between the two experiments was only the application of atrazine; the soil texture and hydrologic regimes were virtually identical. The differences in soil parameters were not statistically significant: Temperature varied by 1.5 °C (due to seasonal variations, despite greenhouse controls), the soil pH varied by 0.1 units, and the redox potential varied by 19 mV. In the first atrazine experiment, a total of 21 µg L^{-1} were added to the plants (High dose) over seven weeks; whereas in this experiment, a total of

42 µg L^{-1} were added twice per month for 28 weeks or 7 months. . However, the frequency of the added doses did not seem to be the difference in the outcome of the two experiments. The persistence of the herbicide in the rhizosphere and inside the plant may be one of the key factors that cause reduced tensile root strength. As a result, this suggests that there is a temporal component to the effects of atrazine and that the impact on *S. patens* does not occur immediately, even though the uptake of atrazine may occur rapidly. Atrazine may be sorbed and desorbed to soil particles and the rate of adsorption and desorption may vary, which is an indication of hysteresis and a lack of equilibrium between the herbicide and the soil and water fractions. However, the addition of nutrients, especially phosphorus, seemed to exacerbate the effects of atrazine exposure on the plants. The atrazine-HP and atrazine-nP units produced the lowest group mean tensile root strengths of the entire experiment (Table 4.1,1.69 ± 0.22 N and 1.76 ± 0.21 N vs. Control at 4.19 ± 0.21 N) and the HP level alone produced the lowest mean tensile strength for an individual treatment (1.32 ± 0.20 N). The effects of the nitrogen-phosphorus combination in concert with atrazine exposure are demonstrated most emphatically by Fig. 4.5(a) and (b). The experimental units clearly lack the biomass of the control and rhizome development was nonexistent. The root biomass may have atrophied because of carbon loss due to respiration as well as curtailed growth due to surplus nutrients and photosynthesis interference by atrazine exposure. The consequences of carbon demand and lack of replenishment of root biomass is manifested by reduced tensile strength. In addition, the lack of rhizome production would have severe biomechanical consequences for the plant and the wetland ecosystem. The plants in Figure 4.6(a)-(d) could be easily uprooted from the soil because of fewer rhizomes and lower fine root production.

(a) 

(b) 

Fig. 4.5 The belowground biomass production for the Control vs. the (a) Medium Atrazine x Low Nitrogen experimental unit, and (b) Medium Atrazine x Low Phosphorus experimental unit in the 212-day atrazine-nutrient interaction experiment. The tensile root strength of the M x LN (2.02 ± 0.23, $p < 0.0001$) was significantly weaker than Control (4.19 ± 0.23); (b) Medium Atrazine x Low Phosphorus experimental unit in the 212-day atrazine-nutrient interaction experiment. The tensile root strength of the M x LP (2.14 ± 0.23, $p < 0.0001$) was significantly weaker than Control (4.19 ± 0.23). Note the lack of rhizomes and decreased number of fine roots on both experimental units. The polygons with the white dotted lines delineate the root biomass present at the beginning of the experiment

The lack of rhizomes on the experimental units may indicate reduced fitness because of the inability of the plant to store photosynthate or produce new ramets. In addition, the lack of rhizomes would severely weaken the biomechanical stability of the plant and soil due to inability to generate new lateral roots with subsequent fine roots and roots hairs. As a result, soil-plant friction would be greatly reduced, which would also decrease the volume of soil that is reinforced by roots and lead to weaker soil shear strength.

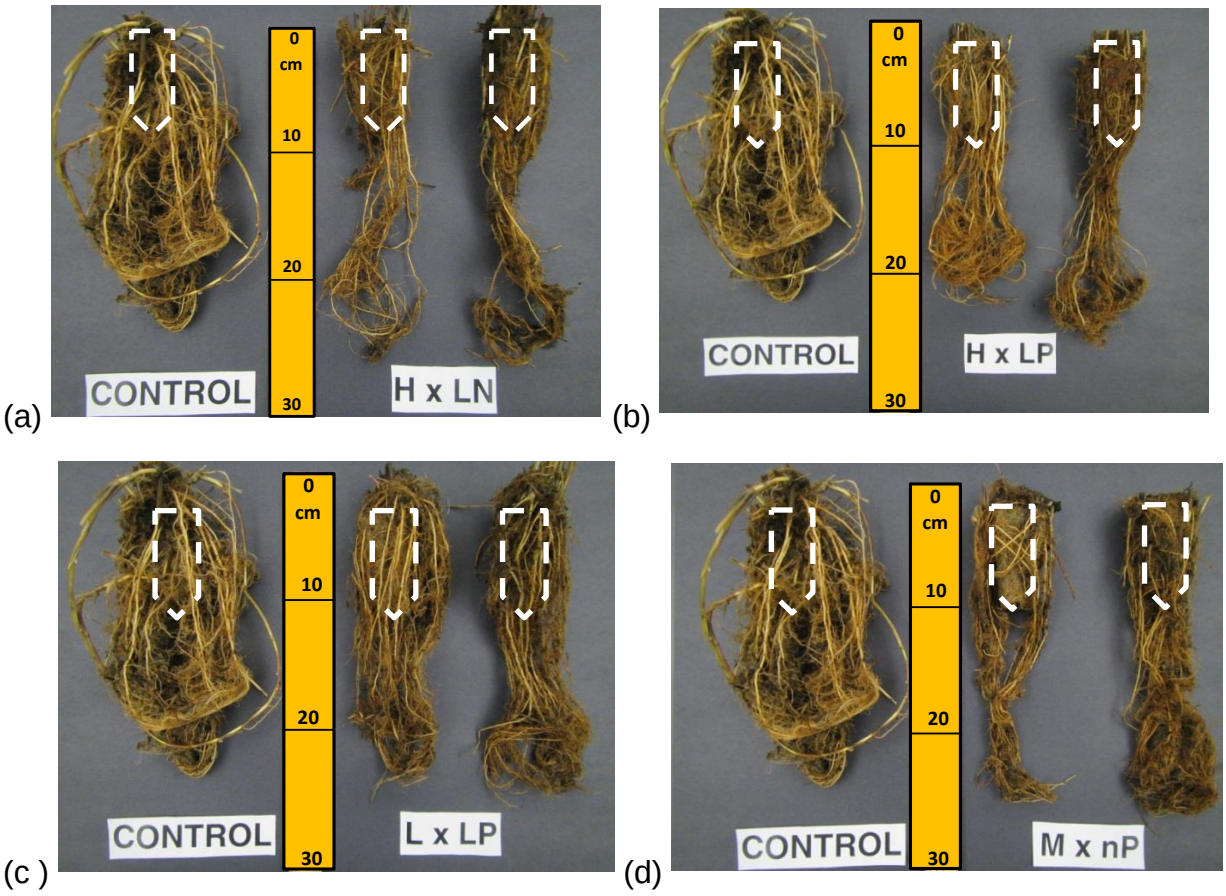


Fig. 4.6 Additional examples of the belowground biomass production for the Control vs. the (a) High Atrazine x Low Nitrogen (H x LN) (b) High Atrazine x Low Phosphorus (H x LP) (c) Low Atrazine x Low Phosphorus (L x LP) (d). Medium Atrazine x Low Nitrogen-High Phosphorus (M x nP) experimental unit in the 212-day atrazine-nutrient interaction experiment. The tensile root strength of both the L x LP (1.66 ± 0.21, $p < 0.0001$) and M x nP (1.54 ± 0.23, $p < 0.0001$) units were significantly weaker than Control (4.19 ± 0.23). Note the lack of rhizomes and decreased number of fine roots on the experimental units. The polygons with the white dotted lines delineate the root biomass present at the beginning of the experiment

The reduced number of roots would result in the additional loading of tensional forces on fewer roots with much less soil-plant friction, which would make them more susceptible to failure. Furthermore, the magnitude of forces need to uproot the plant would be considerably less with the absence of rhizomes and the reduced root architecture.

Interactive Effects

The interactive effects between two substances may be defined as the presence of main effect A affects the activity of main effect B. If the effects of the combination of A and B (AxB) are greater than that of the greater of either A or B, then there are interactive effects of A and B. The Kolmogorov-Smirnov goodness-of-fit test tested the null hypothesis that the distributions of the atrazine-nutrient treatment combination and main effects tensile root strength data were not different. Interactive effects were detected by this test in the High Phosphorus x High Atrazine (HPxH) and Low Phosphorus x High Atrazine (LPxH) treatment combinations. The tensile root strength data distribution of the HPxH (1.32 N) and LPxH (2.82 N) treatment combinations were significantly different from the High Atrazine main effect (1.94 N). However, interactive effects were detected in two out of 18 Kolmogorov-Smirnov tests, which suggest that it is unlikely that there were interactive effects between the six nutrient additions and atrazine doses. In addition, the High Atrazine, HP, and LP main effects reduced the tensile root strength of *S.patens* by 54% (1.94 N), 52% (2.01 N), and 50% (2.11 N), respectively, compared to the Control (4.19 N). The HPxH (1.32 N) and LPxH (2.82 N) treatment combinations reduced the tensile root strength of *S.patens* by 68% and 33%, respectively, compared to the Control (4.19 N). Therefore, the treatment combinations accounted for 16–17% of additional tensile root strength reduction, compared to the main effects. These results suggest that the two main effects had the greatest impact on the tensile root strength of *S. patens* and the 18 treatment combinations had an additive effect on the loss tensile root strength.

Interactive effects between the six nutrient levels and atrazine were not likely present in this experiment. However, it is very important to note that the mean tensile root strengths of the main effects and the nutrient-atrazine treatment combinations were all significantly lower than

the Control and that these treatments and their combinations caused a substantial reduction in the tensile root strength of *S. patens.* In addition, the results of this study have implicated phosphorus as one of the main drivers of belowground biomass degradation of a coastal emergent macrophyte, which is consistent with previous efforts that have produced similar results. These nutrient and atrazine treatments significantly reduced both the belowground biomass and tensile root strength of *S.patens.*

CONCLUSIONS

The tensile root strength of *S. patens* in these experiments declined with exposure to atrazine, nutrients, and in combination with both. The phosphorus and the nitrogen-phosphorus combination had greatest effect on tensile root strength compared to the other treatments. The effects of the nutrient-atrazine combination produced the lowest recorded tensile root strength. The root biomass was visibly smaller than in the controls, and it is highly likely that biomass was lost to respiration or biomass was not regenerated due to the effects of atrazine. The application of atrazine and the addition of nutrients resulted in roots with decreased tensile strength and structurally compromised belowground biomass because of the sparse rhizome and fine root production. The absence of interactive effects with nutrients and atrazine presents a greater management challenge than the presence of interactive effects. If there were interactive effects between nutrients and atrazine, then the stress on coastal plants could be reduced by the removal of one stressor or the other. However, since both nutrients and atrazine severely reduced the tensile root strength of *S. patens*, both toxicants would have to be removed or curtailed to reduce the biomechanical stress on the plants. Coastal macrophytes need the biomechanical reinforcement of roots to resist powerful natural disturbances. In addition, even if the plants are not dislodged from the marsh, the loss in belowground biomass will curtail the wetlands' ability

to accrete new organic matter and keep pace with relative sea level rise. Atrazine has ecosystem-level implications; therefore, beyond the effects it has on the dominant vegetation. As an herbicide, atrazine may also affect phytoplankton and cause ecological damage at other trophic levels, including those of commercially valuable estuarine and marine species. The Louisiana coast receives from agricultural fields in the upper Mississippi River watershed that may affect marine species as well as wetland restoration outcomes within the Holocene floodplain of the Mississippi River.

REFERENCES

Bodker JE, Turner RE, Tweel A, Schulz C, Swarzenski C (2015) Nutrient-enhanced decomposition of plant biomass in a freshwater wetland. Aquatic Botany 127:44-52

Bouldin JL, Farris JL, Moore MT, Smith Jr. S, Cooper CM (2006) Hydroponic uptake of atrazine and lambda-cyhalothrin in *Juncus effusus* and *Ludwigia peploides*. Chemosphere 65:1049-1057

Brady NC, Weil RR (2002) The Nature and Properties of Soils, 13th edition. Prentice Hall Upper Saddle River, New Jersey 960 pp.

Chabreck RH (1972) Vegetation, water, and soil characteristics of the Louisiana coastal region. Bulletin No. 664 Louisiana State University Press, Baton Rouge, LA 72 pp.

Choudhry GG (1983) Humic substances Part III: Sorptive interactions with environmental chemicals. Toxicological and Environmental Chemistry 6(2):127-171

Clay SA, Koskinen WC (1990a) Adsorption and desorption of atrazine, hydroxyatrazine, and s-glutathione atrazine on two soils. Weed Science 38(3):262-266

Clay SA, Koskinen WC (1990b) Characterization of alachlor and atrazine desorption from soils. Weed Science 38(1):74-80

Conley DJ, Schelske CL, Stoermer EF (1993) Modification of the biogeochemical Q5 cycle of silica with eutrophication. Marine Ecology Progress Series 101:179–192

Darby FA, Turner RE (2008a) Below- and aboveground *Spartina alterniflora* production in a Louisiana salt marsh. Estuaries and Coasts 31:223–231

Darby FA, Turner RE (2008b) Below- and aboveground biomass of *Spartina alterniflora*: Response to nutrient addition in a Louisiana salt marsh. Estuaries and Coasts 31(2):326-334

Davis DE, Gramlich JV, Funderburk, HH (1965) Atrazine absorption and degradation by corn, cotton, and soybeans. Weeds 13(3):252-255

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Sergio Fagherazzi S, Wollheim WM (2012) Coastal eutrophication as a driver of salt marsh loss. Nature 490:388-394

D'Elia CF, Sanders JG, Boynton WR (1986) Nutrient enrichment studies in a coastal plain estuary: Phytoplankton growth in large-scale, continuous cultures. Canadian Journal of Fisheries and Aquatic Sciences 43:397-406

Dortch Q, Rabalais NN, Turner RE, Rowe GT (1994) Respiration rates and hypoxia on the Louisiana shelf. Estuaries 17:862–872

Eastin EF, Davis DE (1967) Effects of atrazine and hydroxyatrazine on nitrogen metabolism of selected species. Weeds 15(4):306-309.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: Recent trends, questions and potential solutions. Science 329:889–892

Ghosh PK, Philip L (2006). Environmental significance of atrazine in aqueous systems and its removal by biological processes: An overview. Global NEST Journal 8(2):159-178.

Hoagland DR, Arnon DI (1950) Circular, California Agricultural Experiment Station. 347(2):1-32

Hollis LO, Turner RE (2018) The tensile root strength of five emergent coastal macrophytes. Aquatic Botany (in press)

Justić D, Rabalais NN, Turner RE (1995) Stoichiometric nutrient balance and origin of coastal eutrophication. Marine Pollution Bulletin 30(1):41-46

Justić D, Rabalais NN, Turner RE (1997) Impacts of climate change on net productivity of coastal waters: implications for carbon budgets and hypoxia. Climate Research 8:225-237

Justić D, Rabalais NN, Turner RE (2002) Modeling the impacts of decadal changes in riverine nutrient fluxes on coastal eutrophication near the Mississippi River Delta. Ecological Modelling 152:33–46

Kruger EL, Somasundaram L, Kanwar RS, Coats JR (1993a) Movement and degradation of ^{14}C atrazine in undisturbed soil columns. Environmental Toxicology and Chemistry 12:1969-1975

Kruger EL, Somasundaram L, Kanwar RS, Coats JR (1993b) Persistence and degradation of ^{14}C atrazine and ^{14}C deisopropylatrazine as affected by soil depth and moisture conditions. Environmental Toxicology and Chemistry 12: 1959-1967

Lytle JS, Lytle TF (1998) Atrazine effects on estuarine macrophytes *Spartina alterniflora* and *Juncus roemerianus*. Environmental Toxicology and Chemistry 17(10):1972–1978

Lytle JS, Lytle TF (2005) Growth inhibition as indicator of stress because atrazine following multiple toxicant exposure of the freshwater macrophyte *Juncus effusus* L. Environmental Toxicology and Chemistry 24(5): 1198–1203

Mandelbaum RT, Sadowsky MJ, Wackett LP (2008) Microbial degradation of s-Triazine herbicides *In:* HM LeBaron, JE McFarland, OC Burnside (eds.) The Triazine Herbicides: 50 years Revolutionizing Agriculture. Elsevier, San Diego, CA 601 pp.

McGlamery MD, Slife FW (1966) The adsorption and desorption of atrazine as affected by pH, temperature, and concentration. Weeds (14)3:237-239

Mersie W, Liu J, Seybold C, Tierney D (1998) Extractability and degradation of atrazine in a submerged sediment. Weed Science 46(4):480-486

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago, IL 608 pp.

Niklas KJ, Spatz HC (2012) Plant Physics. University of Chicago Press, Chicago, IL 426 pp.

Nixon SW (1995) Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia 41:199–219

Rabalais NN, Turner RE, Wiseman, Jr. WJ, Dortch Q, Justić D, Sen Gupta BK (1996) Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. Estuaries 19(2B):386-407

Rabalais NN, Turner RE, Wiseman, Jr. WJ (2001) Hypoxia in the Gulf of Mexico. Journal of Environmental Quality 30:320–329

Rabalais NN (2009) Eutrophication of estuarine and coastal ecosystems *In*: R Mitchell, JD Gu (eds.) Environmental Microbiology, Second Edition. John Wiley and Sons, Inc., Hoboken, NJ 624 pp.

Reish DJ, Geesey GG, Kauwling TJ, Wilkes FG, Mearns AJ, Oshida PS, Rossi SS (1980) Marine and estuarine pollution. Journal of the Water Pollution Control Federation 52(6):1980:1533-1575

Reddy KR, Delaune RD (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, New York, NY 774 pp.

Ruddiman W (2013) The Anthropocene. Annual Review of Earth and Planetary Sciences Volume: 41(1):45-68

Seybold C, Mersie W (1996) Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine, and hydroxyatrazine on Levy wetland soil. Journal of Agricultural and Food Chemistry 44:1925-1929

Sharpe SS, Dickens R, Turner DL (1989) Herbicide effects on tensile strength and rooting of Bermudagrass (*Cynodon dactylon*) sod. Weed Technology 3(2):353-357

Turner DL, Dickens R (1987) Atrazine effects on tensile strength of Centipedegrass sod. Agronomy Journal 79:39-42

Turner DL, Sharpe SS, Dickens R (1990) Herbicide effects on tensile strength and rooting of Centipedegrass sod. Horticultural Science 25(5):541-544

Turner RE, Rabalais NN (1994) Coastal eutrophication near the Mississippi River Delta. Nature 368:619-621

Turner RE, Qureshi N, Rabalais NN, Dortch Q, Justić D, Shaw RF, Cope J (1998) Fluctuating silicate:nitrate ratios and coastal plankton food webs. Proceedings of the National Academy of Sciences of the United States of America 95(22):13048-13051

Turner RE, Rabalais NN, Justić D (2008) Gulf of Mexico hypoxia: Alternate states and a legacy. Environmental Science and Technology 42:2323–2327

Turner RE (2011) Beneath the salt marsh canopy: Loss of soil strength with increasing nutrient loads. Estuaries and Coasts 33:1084-1093. doi: 10.1007/s12237-010-9341-y

United States Environmental Protection Agency (USEPA) (2016) Refined Ecological Risk Assessment for Atrazine. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington D.C.

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: Limnology and Oceanography (21)2:245-252

Wigand C, Brennan P, Stolt M, Holt M, Ryba S (2009) Soil respiration rates in coastal marshes subject to increasing watershed nitrogen loads in southern New England, USA. Wetlands 29(3):952–963

Wigand C, Roman CT, Davey E, Stolt M, Johnson R, Hanson A, Watson EB, Moran SB, Cahoon DR, Lynch JC, Rafferty P (2014) Below the disappearing marshes of an urban estuary: historic nitrogen trends and soil structure. Ecological Applications 24(4):633–649

CHAPTER 5
THE TENSILE ROOT STRENGTH OF *SPARTINA PATENS*: RESPONSE TO FLOOD DURATION AND NUTRIENT ADDITION

INTRODUCTION

The timing, frequency, and duration of floods as well as the depth of the floodwaters, can play a pivotal role in the assemblage and trajectory of wetland plant communities and as well as ecosystem functions (Mitsch and Gosselink 2000, Keddy 2010, Cronk and Fennnesy 2001, Willey 2016). Many wetlands are low-gradient basins that may attenuate flood pulses over time due to the long residence time of floodwaters within the wetland basin. However, these flood pulses may have adverse effects on the wetland ecosystem if the residence time is extended beyond the wetland's natural hydropattern.

The flooding and subsequent inundation of a wetland surface severely curtails oxygen diffusion into the soil because the diffusion of gases is 10^4 times slower in water than in the air (Ponnamperuma 1972, 1984; Striker 2012). The decreased oxygen diffusion rate under flooded conditions increases the likelihood of hypoxic conditions developing in the soil. In addition, the biological oxygen demand of soil microbial organisms and the respiration of plant roots may consume the remaining oxygen and induce anoxic conditions. This anoxia creates physiological obstacles for plant survival as soils become flooded. The reduction-oxidation potential (hereafter, redox potential) declines under hypoxic and anoxic conditions, and low redox conditions can lead to the accumulation of phytotoxins such as reduced iron and manganese, hydrogen sulfide, lactic acid, acetaldehyde, ethanol, and formic and acetic acid (Cronk and Fennessy 2001, Fieldler et al. 2007, Striker 2012). Also, flooding can limit and/or halt plant growth because aerobic respiration cannot be sustained under anoxic conditions. However, wetland plants can exhibit numerous physiological, metabolic, and structural adaptations to survive flooded conditions.

The formation of aerenchyma tissue is one anatomical adaptation that helps maintain gas exchange between the belowground biomass of the plant and the atmosphere. Aerenchyma consists of gas-filled spaces called lacunae that lower the resistance of internal gas transport within the plant and facilitate the venting of carbon dioxide, methane, and ethylene to the atmosphere (Cronk and Fennessy 2001, Striker 2012, Willey 2016). Seago et al. (2005) conducted a comprehensive review of aerenchyma formation and described three different processes that lead to aerenchyma tissue: lysigeny, schizogeny, and expansigeny. Lysigeny consists of a reduction in the number of cells due to cell wall separation and the collapse of cells. Striker (2012) identified two distinct patterns of lysigeny: radial lysigeny and tangential lysigeny. In radial lysigeny, lacunae form by the collapse of cells radially aligned in the cortex. The lacunae are separated by intact files of cells or remnants of cell walls. Tangential lysigeny resembles a spider web pattern that is created by cell separation and collapse in tangential sections of the root cortex with intact radial files of cells (Striker 2012, Jung et al. 2008). Schizogeny occurs as a result of the enlargement and separation of cells without cellular collapse (Cronk and Fennessy 2001). Expansigeny involves the formation of lacunae by cell division and enlargement without the cell death that accompanies lysigeny or additional cell wall separation associated with schizogeny (Striker 2012). As aerenchyma tissue forms within the plant organ, such as the stem, leaf, or root, there is an increase in tissue porosity (Burdick 1989, Burdick and Mendelssohn 1987). For instance, in hydrophytes, the total area of lacunae may occupy 50–60% of the total root cross-sectional area (Cronk and Fennessy 2001). As a result, aerenchyma tissue represents an ecological trade-off between maintaining fitness in hypoxic and anoxic soil and the structural integrity of the plant (Puijalon et al. 2007, Puijalon et al. 2008, Puijalon et al. 2011).

The spatial configuration of aerenchyma in the root and rhizome cortex can directly affect the resilience and resistance of the belowground biomass to forces that may be exerted upon the plant. Forces that act on the stem and leaves of plants may be transmitted vertically as tensional and/or compressional forces to the belowground biomass (Niklas 1992, Lamberti-Raverot and Puijalon 2012). Loads that are exerted on the aboveground biomass, such as wave action, wind, water currents, or feeding herbivores, can subject the plant to tensional or uprooting forces. These forces may be exerted upon roots and rhizomes individually or sequentially due to the plant's root morphology and architecture (Puijalon and Bornette 2004, Puijalon and Bornette 2006, Puijalon et al. 2005). Therefore, tensile root strength, which is the resistance of a material to a tensional load, is an important component of a plant's structural integrity. The biomass of roots and rhizomes may be reduced by the formation of aerenchyma under flooded conditions because lacunae occupy a greater percentage of cross-sectional area of the cortex. Four general root structural types have been described based on the spatial configuration of aerenchyma tissue and the arrangement of cells in the cortex: graminaceous, which resembles a bicycle wheel; cyperaceous, which resembles a spider web; a honeycomb pattern attributed to the genus *Rumex*; and an irregular, non-organized structural pattern of aerenchyma attributed to the genus *Apium* (Justin and Armstrong 1987, Seago et al. 2005). Striker et al. (2007) investigated the effects of aerenchyma formation on the mechanical properties of four representative plant species from each structural root type. They found that compressional root strength in *Rumex* and *Apium* decreased with increasing root porosity and root diameter, changes that were attributed to lysigenic processes. Puijalon et al. (2011) demonstrated a negative correlation between the avoidance and tolerance strategies of 28 aquatic plant species that were subjected to mechanical forces generated by water movement. They found that as plants employed an avoidance strategy

to minimize drag forces by adopting a streamlined form, their tolerance strategy to maximize strength to resist breakage was compromised by the avoidance strategy, which produced a physically weaker morphology. Similarly, wetland plants employ aerenchyma tissue formation as an avoidance strategy under anaerobic conditions, but aerenchyma tissue formation may reduce the tolerance of the plant to external mechanical forces that may be exerted by flow.

Flooding events can pose other risks for wetland plants because numerous compounds and substances are transported to wetland environments via water movement. Major flood events often contain large sediment and nutrient loads as well as pesticides, herbicides, petroleum by-products, human personal care products, and other xenobiotics (Reish et al. 1980, Welch et al. 2014). Excess nutrient influxes to coastal wetlands have been implicated as a major driver of wetland loss due to degradation of the belowground biomass. Many researchers have demonstrated that excess nitrogen and phosphorus loads have led to a reduction in belowground biomass (Valiela et al. 1976, Morris and Bradley 1999, Darby and Turner 2008a, Deegan et al. 2012, Graham and Mendelssohn 2014, Graham and Mendelssohn 2016). Also, other studies have demonstrated that nutrient additions have led to higher rates of soil respiration (Morris and Bradley 1999, Wigand et al. 2009) and lower soil strength (Darby and Turner 2008b, Swarzenski et al. 2008, Turner et al. 2009, Turner 2011).

Spartina patens (Ait.) Muhly. is a dominant emergent macrophyte in brackish and intermediate coastal marshes on the Gulf of Mexico and Atlantic coasts of the United States. The presence of this species provides some protection to human communities from flooding and tropical cyclones by delaying flood crest, detaining floodwaters, or attenuating wave action and storm surge (Augustin et al. 2009). In addition, the belowground biomass of *S. patens* provides the biological infrastructure that resist erosional forces by reinforcing wetland soils. Therefore,

understanding the effects of flooding on the structural integrity of this species is important to wetland management in an age of climate change. Although soil shear strength measurements have been a part of numerous studies, the tensile root strength of individual roots subjected to flood conditions and nutrient addition has not been investigated extensively. In addition, few studies, if any, have investigated the interactive effects of flooding and excess nutrient inputs on the mechanical properties of a dominant emergent macrophyte. Here, I describe the results of greenhouse experiments that tested the hypothesis that flood duration and nutrient addition, as well as their interactive effects, reduce the tensile root strength of *S. patens*.

MATERIALS AND METHODS

Flood Duration-Nutrient Addition Interaction Experiment

Plants were grown under natural light conditions in the of the Louisiana State University (LSU) greenhouses at Baton Rouge, LA, USA. The experimental design consisted of a 6 x 2 x 4 factorial design plus four controls with nutrient level and flood duration as the main effects. *Spartina patens* plugs from Tampa Bay Estuary were purchased from the Green Seasons nursery (Tampa, FL). The samples were transplanted to 9.45-L (2.5-gallon) plastic pots filled with 5.5 L of a mixture of 65% sphagnum peat (Premier Sphagnum Peat Moss; 100% Canadian peat moss, no added fertilizer or nutrients), 30% clay/silt mixture, and 5% sand. The transplants were allowed to grow for 16 weeks in greenhouse conditions before beginning the experiment. The sand, silt, and clay components were obtained by the LSU greenhouse staff from soil in the Sterlington soil series (coarse-silty, mixed thermic Typic Hapludalfs) located in the Mississippi River floodplain in West Baton Rouge Parish, LA. The soil texture of clay/silt components was estimated by the texture-by-feel field technique and determined to be sandy clay loam (Brady and Weil 2002). The treatments were rotated monthly during the experiment on a reverse-

orientation basis (e.g. from south to north, and west to east) to reduce the variation in environmental conditions.

The nitrogen and phosphorus nutrient treatments consisted of water soluble granular reagent grade calcium nitrate tetrahydrate [$Ca(NO_3)_2 \bullet 4H_2O$] and granular laboratory grade potassium phosphate [K_3PO_4] (Fisher Scientific; Nazareth, PA). Nutrient treatments, which were added bi-monthly, were: High Nitrogen (HN, 5.0 mg L^{-1}), Low Nitrogen (LN, 1.75 mg L^{-1}), High Phosphorus (HP, 0.30 mg L^{-1}), Low Phosphorus (LP, 0.10 mg L^{-1}), High Nitrogen x Low Phosphorus (Np), and Low Nitrogen x High Phosphorus (nP). The nutrient treatments were added to one liter of deionized water and allowed to dissolve before adding the solution to the experimental treatments.

The flood duration experimental unit set-up consisted of placing each 9.45-L (2.5-gallon) plastic pot inside an 18.9-L (5-gallon) high-density plastic bucket and then filling the bucket with deionized water to 15 cm above the soil surface in the plastic pot. The flood duration treatments were 50% of the designated time frames: Weekly (7 days: 3.5 days flooded, 3.5 days saturated) and Bi-monthly (14 days: 7 days flooded, 7 days saturated). The flood/drained cycle was repeated throughout the experiment. Water levels were manipulated by placing bricks underneath the plastic pots during the drained period and removing the bricks during the flood period. Water levels were maintained ~1.75 cm above the soil surface during the drained phase to ensure saturated soil conditions.

Soil temperature, pH, and redox potential were measured monthly. Soil temperature was measured by a soil probe to the nearest 0.1°C. The pH of the soil pore water was obtained by withdrawing a 175 mL sample of soil pore water with a Lisle vacuum pump (Lisle Corporation, Clarinda, Iowa) and dispensing it into a 250-mL amber glass bottle. The pH was measured with a

Hach HQ 40d multi-parameter meter (Hach Industries Loveland, CO). The redox potential was measured with 45-cm long standard platinum probes following the procedures of Reddy and Delaune (2008) and a Corning calomel reference probe (Corning, Inc. Corning, NY) connected to a Fluke 73 Multimeter (John Fluke Manufacturing, Everett WA). A correction of +244 mV was added to redox measurements to compensate for the difference in redox potential between the calomel probe and standard hydrogen reference electrode (Reddy and Delaune 2008). The experiment lasted for 165 days from 15 November 2015 to 30 May 2016.

Tensile Strength Testing

Tensile strength testing was conducted only on live roots in the small size class (0.5–1.0 mm), because of the high numbers of roots within this diameter range, the increased probability of conducting successful tensile strength tests, and the paucity of dead roots. Six tests were conducted for every successful tensile strength test. A successful test consisted of root samples that failed between the supports of the test stand, whereas roots that failed at the supports were considered unsuccessful tests. Live roots and rhizomes were differentiated from dead roots by their white, turgid, and translucent appearance, whereas dead roots were dark and flaccid (Darby and Turner 2008c). However, many live roots were stained by soil deposits; they were separated from dead roots by the presence of turgor, bifurcations of fine roots, and their ability to float. Three individual root metrics were measured: mass, length, and diameter, while cross-sectional area and volume were calculated from these metrics. Root length was measured with a Scale Master© Classic digital planimeter (Calculated Industries, Carson, NV, USA) to the nearest 0.1 mm. The mean root diameter was measured to the nearest 0.1 mm with a Starrett digital IP67 micrometer. The measurements were taken at both ends and at the middle of each root and then averaged. Cross-sectional area (mm^2) and volume (mm^3) were calculated from length and

diameter measurements after tensile strength testing was performed. Root samples were weighed to the nearest 0.1 mg to estimate individual mass. A Mecmesin MultiTest 1–d motorized stand (Mecmesin Limited; Sinfold, West Sussex, UK) was used to test tensile root strength in Newtons (N). Individual roots were secured to two support clamps aligned perpendicular to the base of the test stand. The contact surfaces of the clamps provided 1.25 x 2.50 cm of area and were lined with fine sandpaper to reduce or eliminate slippage. In addition, the support clamps were attached to a Mecmesin Basic Force Gage load meter, which was capable of measuring 1000 N of force with a precision of 0.1 N. The test stand was activated and the top support was pulled upward by a vertical hydraulic piston until the root exhibited structural failure. The load that induced failure at that point, or breaking force, was recorded as tensile strength.

Tissue Sample Testing

Samples of live leaf and root tissue for each experimental unit and the control were collected after tensile strength testing at the end of the experiment and sent to the LSU Soil Testing and Plant Analysis Laboratory to determine the carbon, nitrogen, and phosphorus tissue content. These results of these tests were used to calculate carbon:nitrogen (C:N) and nitrogen:phosphorus molar ratios (mM g^{-1}).

Statistical Analyses

I conducted an analysis of variance (ANOVA) using JMP v. 13 software (SAS Cary, NC) to test for differences in the mean tensile root strength of the flood duration and nutrient treatment main effects and their respective controls. I used a Tukey-Kramer Honest Significant Difference (HSD) test to detect significant differences between the tensile root strength means. The interactive effects among the main effects were determined by segregating the tensile strength data of one main effect into subsets and then conducting a one-way ANOVA for each

level of the other main effect. The data are reported as the mean ± 1 standard error of the mean unless otherwise noted. Homoscedasticity and normality of residuals were determined with Brown-Forsythe and Shapiro-Wilk tests, respectively. The data that did not meet the assumptions of ANOVA were tested using a Welch's ANOVA, and the differences between the tensile strength means were determined using a Steel-Dwass nonparametric multiple comparison test. Interactive effects of treatment combinations were determined by using a Kolmogorov-Smirnov goodness-of-fit test to compare the data distribution of the combination with that of the strongest main effect of the treatment combination. A Student's *t*-test was used to determine statistical significance between the soil temperature, redox potential, and pH parameter data. The differences among the nutrient and the carbon:nitrogen:phosphorus (CNP) ratios were tested with a one-way ANOVA. All statistical tests were performed at a significance level of $p < 0.05$.

RESULTS

Tensile Root Strength

Details of the tensile root strength responses to the various nutrient and flood duration treatments are discussed next and summarized in Table 5.1 and Table 5.2. A one-way Welch's ANOVA detected significant differences in tensile root strength between all nutrient treatments and Control (Fig. 5.1, $F = 7.6$, $p < 0.0001$); however, there were no significant differences among the tensile root strength of the nutrient treatments. The grand tensile root strength mean was 1.94 ± 0.16 Newtons (N). The mean tensile root strength of the Control (3.25 ± 0.16 N) was 39–50% stronger than the six nutrient treatments.

The tensile root strength between all nutrient treatments and the Control in the Bi-monthly flood duration data subset were significantly different (Fig. 5.2a, $F = 16.4$, $p < 0.0001$). These results seem to suggest that there were interactive effects of nutrient addition and flood

duration on tensile root strength. However, there were no significant differences among the nutrient treatments, and the grand tensile root strength mean was 1.78 ± 0.16 N. Also, the mean tensile root strength of the Control (3.25 ± 0.16 N) was twice that of the HN (1.50 ± 0.16 N), LN (1.62 ± 0.16 N), LP (1.42 ± 0.16 N), Np (1.50 ± 0.16 N), and nP (1.47 ± 0.16 N) treatments. The tensile root strengths of the HP treatments were not significantly greater than the other nutrient treatments (p>0.05), but they were significantly lower than that of the Control treatments (1.70 ± 0.16 N, $p < 0.0001$). Similarly, the tensile root strength between all nutrient treatments and Control in the Weekly flood duration subset were significantly different (Fig. 5.2b, $F = 12.6$, $p < 0.0001$). The mean tensile root strength in the Control treatments (3.25 ± 0.16 N) was twice that of the HN (1.56 ± 0.16 N) and LN (1.60 ± 0.16 N) treatments, but there were no significant differences among the nutrient treatments. The grand tensile root strength mean was 2.02 ± 0.17 N.

A one-way Welch's ANOVA revealed significant differences in the tensile root strength between both flood duration treatments and Control (Fig. 5.3, $F = 18.6$, $p < 0.0001$), and the grand tensile root strength mean was 1.76 ± 0.16 N. However, there was no significant difference in tensile root strength between the Weekly (1.81 ± 0.06 N) and Bi-Monthly (1.53 ± 0.06 N) flood treatments.

There were significant differences in tensile root strength between both flood duration treatments, and the Control in the High Nitrogen data subset (Fig. 5.4a, $F = 25.0$, $p < 0.0001$). However, there were no significant differences between the two flood duration treatments ($p > 0.05$) and the grand tensile root strength mean was 2.10 ± 0.17 N.

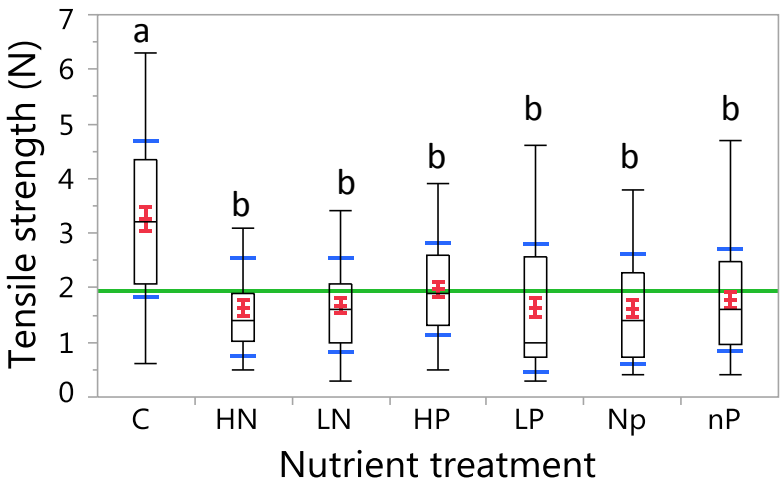


Fig. 5.1 One-way Welch's ANOVA of tensile root strength with nutrient treatment as the main effect for the flood duration-nutrient treatment interaction greenhouse experiment. There were significant differences between control and nutrient treatments ($p < 0.0001$). The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote ± 1 standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments. The treatment abbreviations are: C = control; HN = high nitrogen; LN = low nitrogen; HP = high phosphorus; LP = low phosphorus; Np = high nitrogen + low phosphorus; nP = low nitrogen + high phosphorus treatment

Similarly, a one-way Welch's ANOVA revealed significant differences in tensile root strength between both flood duration treatments and Control in the Low Nitrogen data subset (Fig. 5.4b, F = 22.4, $p < 0.0001$). Also, the Control tensile root strength (3.25 ± 0.17 N) was twice that of the mean tensile root strength in the Weekly (1.59 ± 0.16 N) and Bi-Monthly (1.61 ± 0.16 N) treatments.

The tensile root strength in the two flood duration treatments and Control in the High Phosphorus nutrient treatment data subset were significantly different (Fig. 5.4c, $F = 17.1$, $p < 0.0001$). However, there were no significant differences among the flood duration treatments. The grand tensile root strength mean was 2.34 ± 0.17 N.

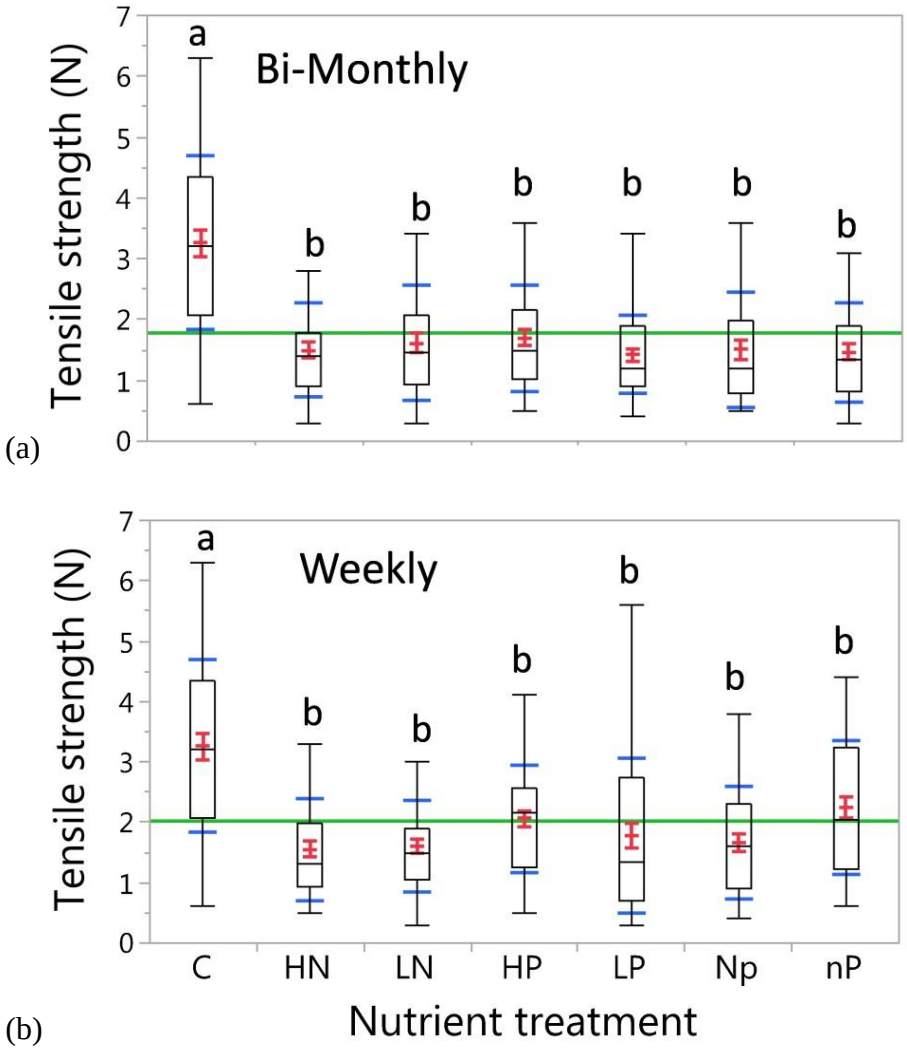


Fig. 5.2 Box-and-whisker plots of one-way Welch's ANOVA of tensile root strength with nutrient treatment as the main effect for the (a) Bi-monthly flood duration and (b) Weekly flood duration data subset to test for interactive effects between nutrient and flood duration treatments. There were significant differences between control and nutrient treatments in both subsets (Table 5.1, $p < 0.0001$), which suggest that there are interactive effects of nutrient addition and flood duration on tensile root strength. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; he center horizontal red lines represent the group mean ± 1 standard error. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments. The treatment abbreviations are the same as in Fig. 5.1

Table 5.1 Summary statistics of the tensile root strength response variable for the nutrient addition and flood duration main effects and main effect subsets (in parentheses) used to test for interactive effects. Statistical significance is indicated by *p*-values < 0.05

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Nutrient	280	n/a	n/a	n/a	1.72	1.94	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.16	1.43	n/a
High Nitrogen (HN)	40	4.4	0.5	1.64	n/a	n/a	0.16	0.89	< 0.0001
Low Nitrogen (LN)	40	4.1	0.3	1.67	n/a	n/a	0.16	0.87	< 0.0001
High Phosphorus (HP)	40	3.9	0.5	1.98	n/a	n/a	0.16	0.85	< 0.0001
Low Phosphorus (LP)	40	4.6	0.3	1.63	n/a	n/a	0.16	1.16	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	5.0	0.4	1.62	n/a	n/a	0.16	1.01	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	4.7	0.4	1.78	n/a	n/a	0.16	0.94	< 0.0001
Nutrient (Weekly)	280	n/a	n/a	n/a	1.82	2.02	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.17	1.43	n/a
High Nitrogen (HN)	40	4.4	0.5	1.56	n/a	n/a	0.17	0.85	< 0.0001
Low Nitrogen (LN)	40	4.1	0.3	1.60	n/a	n/a	0.17	0.77	< 0.0001
High Phosphorus (HP)	40	4.1	0.5	2.06	n/a	n/a	0.17	0.90	< 0.0001
Low Phosphorus (LP)	40	5.6	0.3	1.78	n/a	n/a	0.17	1.29	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	3.8	0.4	1.66	n/a	n/a	0.17	0.94	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	4.4	0.6	2.25	n/a	n/a	0.17	1.12	0.0006

(Table 5.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Nutrient (Bi-Monthly)	280	n/a	n/a	n/a	1.54	1.78	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.15	1.43	n/a
High Nitrogen (HN)	40	3.5	0.3	1.50	n/a	n/a	0.15	0.78	< 0.0001
Low Nitrogen (LN)	40	4.6	0.3	1.62	n/a	n/a	0.15	0.95	< 0.0001
High Phosphorus (HP)	40	3.6	0.5	1.70	n/a	n/a	0.15	0.87	< 0.0001
Low Phosphorus (LP)	40	3.4	0.4	1.42	n/a	n/a	0.15	0.64	< 0.0001
High Nitrogen-Low Phosphorus (Np)	40	5.0	0.5	1.50	n/a	n/a	0.15	0.95	< 0.0001
Low Nitrogen-High Phosphorus (nP)	40	3.8	0.3	1.47	n/a	n/a	0.15	0.82	< 0.0001

(Table 5.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Flood Duration	120	n/a	n/a	n/a	1.85	2.31	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.16	1.43	n/a
Weekly	40	4.4	0.5	2.03	n/a	n/a	0.16	1.01	< 0.0001
Bi-Monthly	40	3.5	0.4	1.66	n/a	n/a	0.16	0.84	< 0.0001
Flood Duration (HN)	120	n/a	n/a	n/a	1.53	2.10	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.16	1.43	n/a
Weekly	40	4.4	0.5	1.56	n/a	n/a	0.16	0.85	< 0.0001
Bi-Monthly	40	3.5	0.3	1.50	n/a	n/a	0.16	0.78	< 0.0001
Flood Duration (LN)	120	n/a	n/a	n/a	1.61	2.16	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.17	1.43	n/a
Weekly	40	4.1	0.3	1.60	n/a	n/a	0.17	0.77	< 0.0001
Bi-Monthly	40	4.6	0.3	1.62	n/a	n/a	0.17	0.95	< 0.0001
Flood Duration (HP)	120	n/a	n/a	n/a	1.88	2.34	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.17	1.43	n/a
Weekly	40	4.1	0.3	2.06	n/a	n/a	0.17	0.90	< 0.0001
Bi-Monthly	40	4.6	0.3	1.70	n/a	n/a	0.17	0.87	< 0.0001

(Table 5.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Flood Duration (LP)	120	n/a	n/a	n/a	1.60	2.15	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.19	1.43	n/a
Weekly	40	4.1	0.3	1.78	n/a	n/a	0.19	1.29	< 0.0001
Bi-Monthly	40	4.6	0.3	1.42	n/a	n/a	0.19	0.64	< 0.0001
Flood Duration (Np)	120	n/a	n/a	n/a	1.57	2.14	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.18	1.43	n/a
Weekly	40	4.1	0.3	1.66	n/a	n/a	0.18	0.94	< 0.0001
Bi-Monthly	40	4.6	0.3	1.50	n/a	n/a	0.18	0.95	< 0.0001
Flood Duration (nP)	120	n/a	n/a	n/a	1.86	2.32	n/a	n/a	< 0.0001
Control	40	6.3	0.6	3.25	n/a	n/a	0.18	1.43	n/a
Weekly	40	4.4	0.6	2.25	n/a	n/a	0.18	1.12	< 0.0001
Bi-Monthly	40	3.8	0.3	1.47	n/a	n/a	0.18	0.82	< 0.0001

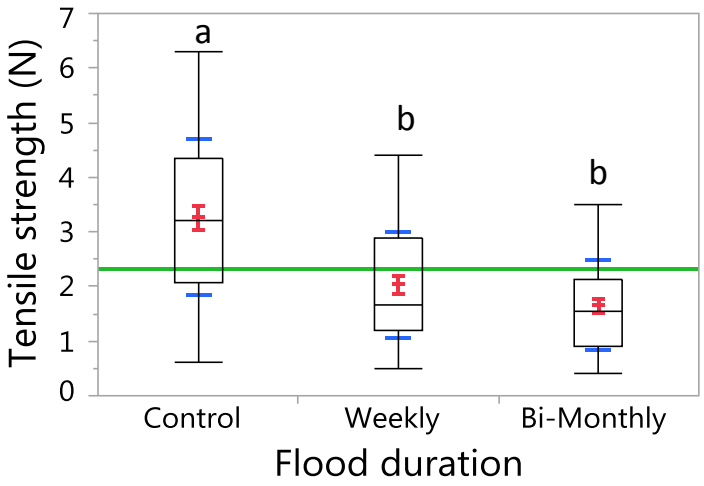


Fig. 5.3 One-way Welch's ANOVA of tensile root strength with flood duration as the main effect for the flood duration-nutrient treatment interaction greenhouse experiment. There were significant differences between control and flood duration treatments ($p < 0.0001$); but there were no significant differences between the Weekly and Bi-Monthly treatments ($p > 0.05$). The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote ± 1 standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments

The tensile root strength between the two flood duration treatments and Control in the Low Phosphorus nutrient treatment data subset were significantly different (Fig. 5.4d, $F = 27.1$, $p < 0.0001$), but there were no significant differences among the flood duration treatments. The grand tensile root strength mean was 2.15 ± 0.17 N.

The results from a one-way Welch's ANOVA revealed significant differences in tensile root strength between both flood duration treatments and Control in the High Nitrogen-Low Phosphorus data subset (Fig. 5.4e, $F = 22.5$ $p < 0.0001$), and the grand tensile root strength mean was 2.14 ± 0.18 N. Similarly, the tensile root strength between the two flood duration treatments and Control in the Low Nitrogen-High Phosphorus nutrient treatment data subset were significantly different (Fig. 5.4f, $F = 24.6$, $p < 0.0001$). The grand tensile root strength mean was 2.32 ± 0.18 N.

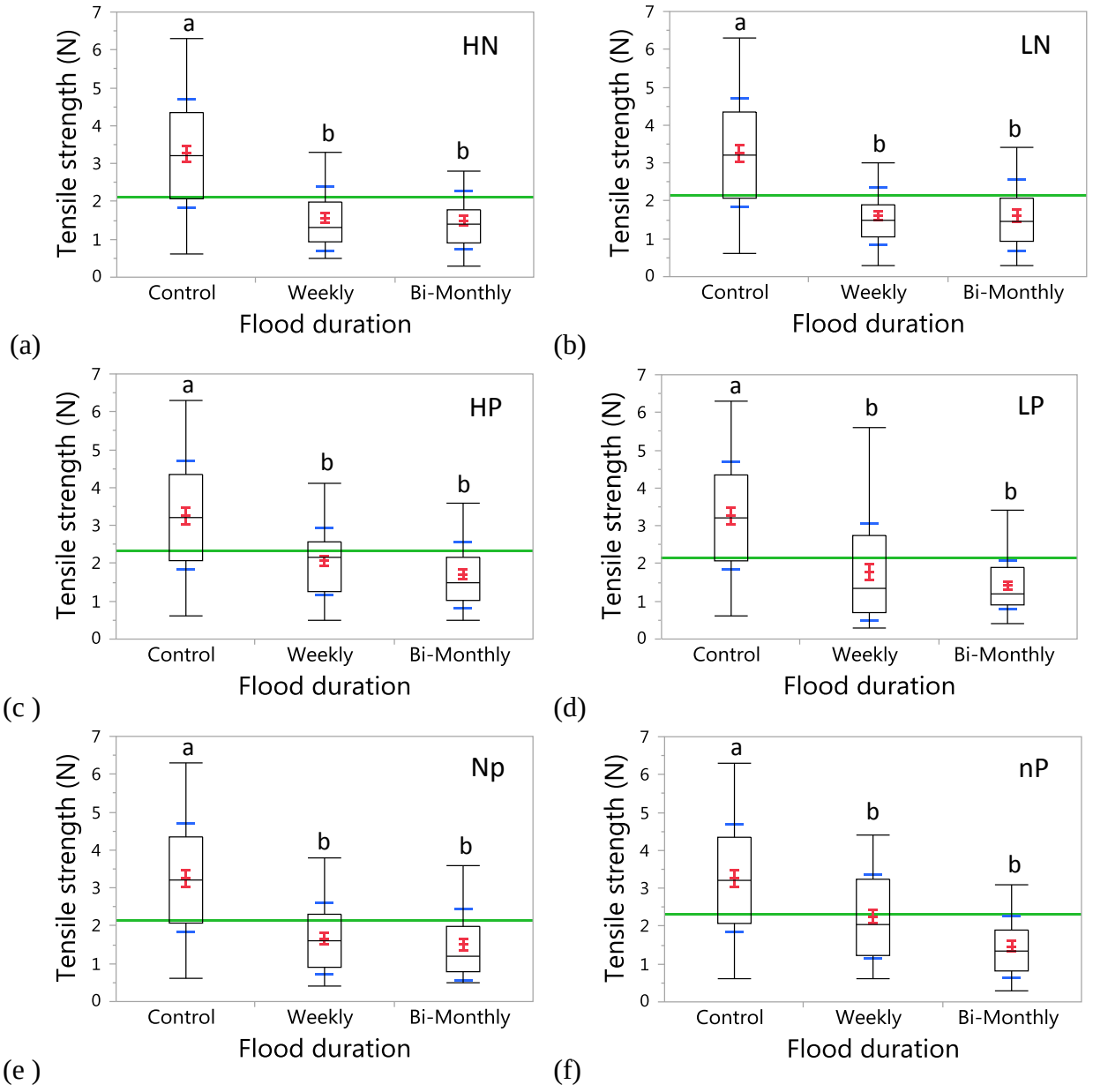


Fig. 5.4 One-way Welch's ANOVA of tensile root strength with flood duration as the main effect for the six nutrient data subsets to test for interactive effects between nutrient and flood duration treatments. There were significant differences between control and flood duration treatments ($p < 0.0001$), which suggest that there are interactive effects of nutrient addition and flood duration on tensile root strength. The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote ± 1 standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote significant differences between treatments. The treatment abbreviations are the same as in Fig. 5.1

A Kolmogorov-Smirnov goodness-of-fit test was conducted to determine the presence of interactive effects between the flood duration and nutrient main effects. The results of the test indicated that there were no significant differences between the data distributions of the main effects and that of the combination treatments. As a result, no interactive effects were detected between the two main effects.

Table 5.2 Summary of one-way Welch's ANOVA tests of the tensile root strength response variable for the nutrient addition and flood duration main effects and main effect subsets (in parentheses) testing for interactive effects. Statistical significance is indicated by *p*-values < 0.05. The treatment abbreviations are the same as in Fig. 5.1

Source	[1]DFNum	[2]DFDen	F Ratio	*p*-value
Nutrient	6	121.1	7.6	< 0.0001
Nutrient (Weekly)	6	108.2	15.9	< 0.0001
Nutrient (Bi-Monthly)	6	107.3	16.4	< 0.0001
Flood Duration	2	74.9	18.6	< 0.0001
Flood Duration (HN)	2	74.7	25.0	< 0.0001
Flood Duration (LN)	2	74.2	22.4	< 0.0001
Flood Duration (HP)	2	75.5	17.1	< 0.0001
Flood Duration (LP)	2	68.3	27.1	< 0.0001
Flood Duration (Np)	2	76.0	22.5	< 0.0001
Flood Duration (nP)	2	74.2	24.6	< 0.0001

[1]Degrees of Freedom -Numerator; [2]Degrees of Freedom - Denominator

Soil Parameters

The mean soil temperature in the experimental treatments ranged from 26.1 to 26.6°C (Table 5.3; Appendix C, Fig. C1) with an overall mean of 26.3 ± 0.41 °C. A Student's *t*-test revealed no significant difference tensile root strength among the soil temperatures or between the two flood duration treatments and Control treatments ($p > 0.05$). The pH of the experimental treatments was neutral to alkaline throughout the experiment and the mean pH was 7.1 in both flood duration treatments and the Control (Table 5.3; Appendix C, Fig. C2).

Table 5.3 Summary of mean soil parameters of a nutrient-flood duration interaction experiment delineated by flood duration treatment. Mean values with different letter superscripts are significantly different ($p < 0.05$)

Parameter	Experimental Treatments		
	Weekly	**Bi-Monthly**	**Control**
Soil Temperature (°C)			
Mean	25.1[a]	25.1[a]	24.9[a]
Min	23.9	23.9	26.4
Max	26.5	26.5	26.4
Standard Error	0.16	0.15	0.19
pH			
Mean	6.6[a]	6.7[a]	6.6[a]
Min	6.5	6.4	6.4
Max	6.7	6.8	6.8
Standard Error	0.01	0.03	0.03
Redox Potential (mV)			
Mean	39.7[a]	37.5[a]	39.8[a]
Min	33.4	25.6	19.2
Max	50.9	44.3	55.1
Standard Error	1.2	1.2	2.3

As a result, a Student's t-test found no significant differences between the soil pH between the flood duration treatments and the Control ($p > 0.05$).

The redox potential fluctuated frequently between the experimental treatments throughout the duration of the experiment. There was less than 6 mV of variation between the redox potential means of the experimental treatments and Control (Table 5.3; Appendix C, Fig. C3). Consequently, a Student's *t*-test revealed no significant differences in the soil redox potential between the flood duration treatments and Control ($p > 0.05$).

Plant Tissue Nutrient Content

The carbon content for the nutrient treatments in the roots was higher than in the Control, with the exception of the Low Nitrogen (LN) treatment (ANOVA, $p < 0.05$; Table 5.4). In

addition, a greater concentration of carbon was detected in the roots than in the aboveground tissue. In the aboveground tissue, the carbon content in the High Nitrogen-Low Phosphorus (Np) treatment was significantly different from the Low Phosphorus (LP) and the LN treatments (p = 0.0039 and p = 0.0032, respectively). There were greater concentrations of nitrogen and phosphorus in the roots than in the stems. The nitrogen content of the nutrient treatment treatments in both the roots and stem was higher than the Control; however there was no significant difference in nitrogen content between the aboveground and belowground tissue and Control ($p > 0.05$). The phosphorus content of the nutrient treatment treatments in both the roots and stem was also higher than the Control; however there was no significant difference in phosphorus content between the aboveground and belowground tissue and Control ($p > 0.05$).However, a Student's *t*-test found that there was a significant difference in the phosphorus content between the nutrient treatment treatments and controls in roots and in the nutrient treatment treatments and Controls in the stem ($p < 0.05$).

The C:N ratio for both the roots and the stems were less than 100 (Table 5.4). The C:N ratio of the roots ranged from 66.1 in the HN treatments to 83.4 in the Control; whereas the C:N ratio in the stem ranged from 72 in the HN treatments to 84 in the Control. In addition, the C:N ratio of the aboveground nutrient treatments was higher than those of the belowground nutrient treatment treatments. However, in the N:P ratios, the stem ratios in the nutrient treatment treatments were higher than the root ratios. The N:P ratios of the roots ranged from 8.9 in the LN treatments to 14.7 in Control treatments. The N:P ratios of the stems ranged from 14.3 in the HP treatments to 23.5 in the Control.

Table 5.4 Results of nutrient tissue content testing of live *S. patens* above- (stem) and belowground biomass (roots) for carbon, nitrogen, and phosphorus as well as carbon-nitrogen (C:N) and nitrogen-phosphorus (N:P) ratios. Mean values with different letter superscripts are significantly different (p <0.05). Comparisons of means were made within each nutrient between treatments and control as well as between roots and stems

Treatment	**Carbon (mmol/g)**		**Nitrogen (mmol/g)**		**Phosphorus (mmol/g)**		**C:N**		**N:P**	
	Roots	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*
HN	37042^{a}	35481^{d}	560.2^{b}	492.5^{c}	52.1^{c}	26.7^{e}	66.1	72.0	10.8	18.4
LN	35890^{a}	36087^{c}	508.0^{b}	478.8^{c}	57.3^{c}	31.5^{e}	62.9	75.4	8.9	15.2
HP	38121^{a}	35879^{c}	541.6^{b}	458.3^{c}	40.8^{ad}	32.1^{e}	70.4	78.3	13.2	14.3
LP	38119^{a}	36080^{c}	510.4^{b}	452.7^{c}	46.1^{ac}	31.3^{e}	74.7	79.7	11.1	14.5
Np	38029^{a}	35213^{d}	550.3^{b}	437.5^{c}	45.2^{acd}	28.0^{be}	69.1	80.5	12.2	15.6
nP	37440^{a}	35682^{d}	515.6^{b}	453.2^{c}	42.9^{ad}	30.1^{e}	72.6	78.7	12.0	15.1
Control	36595^{a}	35966^{cd}	439.0^{a}	428.2^{a}	29.9^{a}	18.2^{b}	83.4	84.0	14.7	23.5

DISCUSSION

S. patens responded to the increased flood duration and nutrient additions with reduced tensile root strength. The six nutrient treatments and both flood duration treatments produced tensile root strengths that were 43 to 47% less than that of the Control treatments, while the combination treatments were 42 to 53% less than that of the Control treatments. In all cases, the differences were statistically significant at $p < 0.05$ (Table 5.1). The main experimental and combination treatment effects may have weakened the belowground biomass of *S. patens* due plant adaptation to the 1) treatments, 2) biogeochemical cycling, or 3) physiological stress. These possibilities are discussed next.

Tensile Root Strength

The tensile root strength was lower in the six nutrient treatments than in the Control treatments ($F = 7.6$, $p < 0.0001$), and was lowest in the HN, LP, and Np treatments of all six (1.64 ± 0.16, 1.63 ± 0.16, and 1.62 ± 0.16 N, respectively). An increased nitrogen loading to coastal wetlands has frequently been implicated in the degradation of the belowground biomass of wetland macrophytes, but has also resulted in increased growth of the aboveground biomass. The plant roots provide respiratory tissue and facultative anaerobic bacteria can utilize nitrate as a terminal electron acceptor to oxidize organic carbon within the roots. As NO_3^- is reduced to either N_2O or N_2 gas, a trade-off occurs as the structure of the root is weakened in order to facilitate energy production. Plant cell walls consist of a matrix of cellulose-rich macrofibrils that are embedded in an amorphous matrix comprised of non-cellulosic carbohydrates (Esau 1977). The plant cell walls are deposited in layers, with the older primary cell wall in contact with the middle lamella, which is a pectinaceous layer binding adjacent cells together (Niklas 1992). During respiration or denitrification, the labile root tissue in the amorphous matrix is most likely

to be used first as an electron donor. As a result, the cell wall may be weakened as the amorphous matrix and the middle lamella are degraded during the oxidation of organic carbon. Tensile forces impacting the cell wall can be transmitted throughout the wall. If the matrix of the cell wall is degraded, then the force of an external load may be distributed among the macrofibrils. When cell walls fail under tension, then the failure usually begins at the middle lamella and the fractures propagate across the cell wall and lead to tissue rupture (Niklas 1992, Niklas and Spatz 2012). The tensile root strength of the HP and nP treatments were higher (1.98 ± 0.16 and 1.78 ± 0.16 N, respectively) than in the other treatments (< 1.67 N). This may have occurred because use of nitrate as an electron acceptor to oxidize the labile components may have weakened the root cell walls. However, phosphorus is not removed from a wetland system by redox reactions like nitrogen, and the accumulation of phosphorus in the soil and plant tissue can also affect tensile root strength.

Excess phosphorus additions, for example, can affect root growth by curtailing root foraging for nutrients. One tenet of the marginal value theorem of the optimum foraging theory states that an organism will spend more time foraging in a resource-rich patch ("Giving-Up Time" GUT) than in a resource-poor patch. McNickle and Cahill (2009) confirmed their hypothesis that plant roots would stop growing at the edges of a nutrient-enriched patch until the value of that patch had been greatly reduced. A prevalent idea about weak wetland soils is that soil strength is diminished because of the loss of belowground biomass to processes such as microbial respiration and denitrification. However, it is entirely possible that belowground biomass may not only be lost, but it may be lost *and not replaced*, i.e. the roots stop growing because there is a surfeit of nutrients to meet their metabolic needs. The loss of biomass within the roots may result in reduced tensile root strength. In this study, the LP treatment resulted in a

mean tensile root strength of 1.63 ± 0.16 N, which was 50% less than that of the Control (3.25 ± 0.16 N). Although nitrogen can increase microbial respiration rates or facilitate carbon loss via denitrification, phosphorus may impact the tensile root strength of wetland macrophytes simply by being present in amounts that exceed the needs of the plant. In addition, an overabundance of phosphorus can also cause a shift in microbial communities, which would directly affect soil biogeochemical cycling. Simultaneous additions of nitrogen and phosphorus can have synergistic effects on the belowground biomass. The Np treatment in this study produced a mean tensile root strength of 1.62 ± 0.16 N vs. 3.25 ± 0.15 N in the Control treatments; whereas the HP treatment had a mean tensile root strength of 1.98 ± 0.16 N. Sundareshwar et al. (2003) reported that nitrogen and phosphorus combination treatments increased soil respiration rates and carbon turnover in a South Carolina salt marsh. Darby and Turner (2008a) also reported a decline in root biomass with nitrogen-phosphorus combination treatments in Louisiana salt marshes and they documented that root foraging decreased with only the increase in phosphorus availability. The availability of nitrogen and phosphorus and subsequent resource partitioning in plant tissue may be elucidated by examining nutrient ratios. The allocation of resources within the plant can provide evidence that may explain the variance in tensile root strength as a function of both nutrient availability and quality.

Reddy and Delaune (2008) reported that the C:N ratio can be used to ascertain the immobilization and mineralization of nitrogen and predict the decomposition rate of plant detritus by microorganisms (Reddy and Delaune 2008). They stated that if the C:N ratio is greater than 100, then immobilization occurs; conversely, if the C:N ratio is less than 100, then ammonium ions will be released during microbial decomposition. The C:N ratios of the six nutrient treatments in these experiments were higher in the stems (85.8 to 100.9) than in the roots

(59.8 to 79.8). The higher C:N ratio in the stems may be an indication of nitrogen immobilization, whereas the lower ratio in the roots suggests that nitrogen mineralization was more likely, albeit at a slow rate.

The tensile root strength may also be affected by the components of organic matter. Labile components such as proteins and carbohydrates are decomposed readily, whereas the more refractory elements such as hemicellulose, cellulose, and lignin are degraded more slowly. Recall from the introduction that the more refractory elements of root tissue reside in the secondary cell wall and that the macrofibrils reside in the primary cell wall. The carbon in plant detritus with a higher C:N ratio may be lost as carbon dioxide during microbial decomposition (Reddy and Delaune 2008); therefore the C:N ratios in the *S. patens* roots suggest that there may have been some alteration of the internal structure, which resulted in lower tensile root strength. The higher C:N ratio in the stems also suggests that nitrogen supply was allocated to the aboveground biomass. The N:P ratio, however, can be used to discern which of the two macronutrients are limiting to plant growth. The molar N:P ratios of the six nutrient treatments in the stems and roots did not vary greatly. The root N:P ratios ranged from 7.2 (HP) to 10.6 (Control) and the stem N:P ratios ranged from 9.6 (HN and LN) to 11.3 (nP). It appears, therefore, that nitrogen was the limiting nutrient in both the stems and the roots. However, nitrogen may not have limited plant growth; the N:P ratio may have been distorted by the higher concentration of phosphorus in the roots vs. in the Control treatments. The nitrogen and phosphorus plant levels represent the amount of nutrients present in the plant prior to the experiment, as well as nutrients absorbed from the soil medium and the nutrient treatments. The N:P ratios indicate that phosphorus was present in concentrations that were sufficient to affect

tensile root strength by stopping or curtailing root growth and proliferation, whereas nitrogen acted as an electron acceptor to facilitate the loss of carbon during respiration.

The two flood duration treatments had significant effects on the tensile root strength of *S. patens*. The Bi-Monthly flood duration treatment resulted in a lower tensile root strength than the Weekly treatment (Fig 5.4), an indication that the tensile root strength declined as the number of flood days increased, but not how quickly. The difference in tensile strength between the two treatments may be due the extent of aerenchyma formation in the roots, which progresses as flood duration continues. In addition, the Bi-Monthly flood duration treatment probably had a stronger effect on tensile root strength because of the capacity of flood conditions to rapidly and drastically alter the internal structure of the roots. In other words, the porosity of the roots may increase as flood duration increases. For example, the ranges of the redox potentials (20 to 55 mV) indicate that the formation of aerenchyma tissue was a likely occurrence. As a result, the root porosity increased with the number of lacunae, which led to a decrease in load-bearing tissue and resulted in lower tensile root strength. Also, the formation of additional aerenchyma tissue can occur and increase root porosity if the redox potential continues to decrease. Stomatal closure during flood conditions can reduce the rate of photosynthesis, which could prevent the plant from fixing carbon to generate new roots. Hypoxic conditions within the roots are a precursor to the generation of ethylene, which exacts a carbon demand on the plant that could further weaken the internal structure of the roots, *in addition* to the formation of lacunae and aerenchyma. The process for the formation of aerenchyma tissue in the roots can begin within hours after the soil is inundated. As oxygen is depleted from the soil and anoxic conditions form, highly developed aerenchyma tissue increases root porosity and decreases structural integrity. Hydrophytes, especially coastal macrophytes, have adapted to stressors of the estuarine

environment, which can include mechanical forces. The formation of aerenchyma tissue is an ecological trade-off that allows wetland plants to increase their fitness in saturated soil conditions but leads to a vulnerability to mechanical forces due to the reduced tensile root strength of aerenchymatous tissue. Anaerobic soil conditions occur every day in coastal wetlands, whereas impacts from tropical cyclones may not occur for years. Therefore, the continuation of gas exchange to stave off asphyxiation and maintain metabolic functions seems to be the more pressing survival issues for wetland plants.

Jung et al. (2008) reviewed the anatomical patterns of aerenchyma and found that radial lysigeny was the most frequent form in the Family Poaceae. The radial lysigeny process produces aerenchyma tissue by schizogenous cell wall separations that are followed by the death and collapse of cells along radial sectors of the middle cortex (Seago et al. 2005, Soukup et al. 2002). The aerenchyma tissue created by radial lysigeny resembles a bicycle wheel with numerous spokes. (not to be confused with the 'wheel-shaped schizogeny' description by Seago et al. 2005). As a result, the increased formation of aerenchyma will result in a greater number of structural junctions, which are much more abundant between the endodermis around the vascular cylinder and the epidermis. Any tensile load applied to the root will be transmitted along the thinner aerenchyma tissue. However, Niklas (1992) recommended that for biomechanical purposes, plant tissue should be treated like structures with multiple components. Other researchers have suggested that variants of aerenchyma tissue are "structurally complex but mechanically efficient, strength being preserved with a minimum of respiratory demand and gas-flow impedance" (Jackson and Armstrong 1999, p. 278); however, these researchers, to my knowledge, did not test the tensile strength of plant biomass subjected to xenobiotics or

prolonged, natural environmental stressors such as flood duration and frequency (Arber 1920, Sculthorpe 1967, Justin and Armstrong 1987, Jung et al. 2008).

The increased porosity created by aerenchyma tissue is not the only biomechanical hazard. Flooding induces the formation of ethylene, which plays an important role in aerenchyma formation. Ethanol is formed as a by-product of anoxic conditions in the soil, which induces anaerobic respiration. Both ethylene and ethanol are formed by the plant and their production levies a carbon cost upon the generating tissue. As flood duration continues, root biomass may therefore be lost due to the production of plant hormones. Also, flooding can induce the closure of the stomata, which leads to a reduction or complete shutdown of photosynthesis. Consequently, the growth of plant roots may be inhibited by a reduction or interruption of photosynthesis. Visser and Sandy (2009) found that *S.patens* displayed a rapid decline in biomass with increased flooding during a 7-week mesocosm experiment. They found that the lowest biomass values in treatments that were flooded over 50% of the time. These results are consistent with the results of this study in that a significant effect of flooding was observed at the 50% flood duration time interval. Slower or nonexistent root growth may lead to reduced uptake of essential nutrients, which creates a positive feedback because an inadequate nutrient supply will also curtail root growth. Furthermore, nutrient cycling processes such as denitrification can exert an additional carbon demand on the roots, which may weaken structural integrity.

Interactive Effects

The Kolmogorov-Smirnov goodness-of-fit tests compared the tensile root strength data distributions of the flood duration and nutrient main effects with those of the flood-duration-nutrient combination treatments. The results revealed that there were no differences between the

data distributions of main effects and combination treatments. Therefore, there were no significant interactive effects between the flood duration and nutrient addition main effects on the tensile root strength of *S. patens*.

CONCLUSIONS

The tensile root strength of *S. patens* was significantly weakened by nutrient addition and increased flood duration, with a greater effect of flood duration than nutrient addition. However, the added nutrients also appeared to have curtailed root growth. Flooding creates hypoxic and/or anoxic conditions in the rhizosphere, which induces the plant to initiate several morphological, anatomical, and physiological adaptations necessary to survive a stressful environment. Aerenchyma helps maintain gas exchange, aerate the roots, and reduce oxygen demand, but its formation entails a trade-off between short-term and long-term fitness. An extended flood duration has long been recognized as detrimental to emergent macrophytes. The formation of aerenchyma significantly reduces tensile root strength over time as flood conditions persist. The formation of aerenchyma tissue is one adaptation to flooding stress and occurs within hours of flooding and can progress as the redox potential decreases and the ethylene concentration within the root increases. This aerenchyma formation results in the loss of tensile root strength as flood conditions persist. However, frequent periods of prolonged flood duration appear to inflict chronic stress upon *S. patens* that exacts a carbon cost and reduces biomass. The plants are able to meet their metabolic needs in the short-term, but the reduction in root biomass reduces tensile root strength and compromises long-term ecological fitness as the marsh becomes vulnerable to natural and anthropogenic biomechanical forces. These indirect impacts on tensile root strength are subtle, perhaps exposed in unusual events, and should be a factor of concern for species with similar ecological adaptations.

Flood waters may be a source of pollutants and alternate electron acceptors in anaerobic, carbon-rich environments, which may increase plant stress. The cumulative effect of these stressors on coastal wetlands is that the impaired and weakened belowground biomass is more vulnerable to erosive threats including higher storm surge, increased flooding, and stronger currents and wind. These chemical stressors are often from non-point pollution, of course. Wetland restoration is a reasonable means to indirectly reduce this non-point source. This includes well-known hydrologic restoration through backfilling of dredged canals, levees reduction, and wetland restoration that facilitates more natural inundation and drainage. However, the absence of interactive effects indicates that both stressors must be managed in order to reduce the negative impacts on wetland plants. Flood events are natural part of the wetland hydropattern; however, flood events of anthropogenic origin such as the alteration of natural hydrology with flood control infrastructure or water diversion projects, can increase stress on wetland plants if the plants are excessively inundated or the residence time of floodwaters is prolonged. Also, these processes may be exacerbated by climate change if the occurrence of tropical cyclones and precipitation events are more frequent.

The existence of coastal marshes dominated by *Spartina* spp. provides ample evidence of the resilience of the species to biomechanical forces generated by natural stressors; but, this study has indicated that over time, the *prolonged exposure to natural stressors and xenobiotics* can weaken the tensile root strength of a coastal macrophyte, reduce its ecological fitness, and increase its vulnerability to high energy disturbances that can lead to uprooting and marsh erosion.

REFERENCES

Arber A (1920) The vegetative morphology of *Pistia* and the Lemnaceae. Proceedings of the Royal Society of London. Series B, containing papers of a biological character 91(636):96-103

Augustin LN, Irish JL, Lynett P (2009) Laboratory and numerical studies of wave damping by emergent and near-emergent wetland vegetation. Coastal Engineering 56:332–340

Brady NC, Weil RR (2002) The Nature and Properties of Soils, 13th edition. Prentice Hall Upper Saddle River, New Jersey 960 pp.

Burdick DM, Mendelssohn IA (1987) Waterlogging responses in dune, swale and marsh populations of Spartina patens under field conditions. Oecologia 74(3): 321-329

Burdick DM (1989) Root aerenchyma development in Spartina patens in response to flooding. American Journal of Botany 76(5):777-780

Cronk JK, Fennessy MS (2001) Wetland Plants: Biology and Ecology. CRC Press, New York, NY 462 pp.

Darby FA, Turner RE (2008a) Below- and aboveground biomass of *Spartina alterniflora*: Response to nutrient addition in a Louisiana salt marsh. Estuaries and Coasts 31(2):326-334

Darby FA, Turner RE (2008b) Effects of eutrophication on salt marsh root and rhizome biomass accumulation. Marine Ecology Progress Series 363:63–70

Darby FA, Turner RE (2008c) Below-and aboveground *Spartina alterniflora* production in a Louisiana salt marsh. Estuaries and Coasts 31(1):223–231

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM (2012) Coastal eutrophication as a driver of salt marsh loss. Nature 490:388-392

Esau K (1977) Anatomy of Seed Plants, Second Edition. John Wiley and Sons, Inc. New York, NY 550 pp.

Fiedler S, Vepraskas MJ, Richardson JL (2007). Soil redox potential: importance, field measurements, and observations. Advances in Agronomy 94:2–56

Graham SA, Mendelssohn IA (2014). Multiple levels of nitrogen applied to an oligohaline marsh identify a plant community response sequence to eutrophication. Marine Ecology Progress Series 417:73–82

Graham SA, Mendelssohn IA (2016). Contrasting effects of nutrient enrichment on below-ground biomass in coastal wetlands. Journal of Ecology 104:249–260

Jackson MB, Armstrong W, (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biology 1:274–287

Jung JD, Lee SC, Choi HK (2008) Anatomical patterns of aerenchyma in aquatic and wetland plants. Journal of Plant Biology 51(6):428-439

Justin SHFW, Armstrong W (1987) The anatomical characteristics of roots and plant response to soil flooding. New Phytologist 106:465–495

Keddy PA (2010) Wetland Ecology: Principles and Conservation. Cambridge University Press, New York, NY 497 pp.

Lamberti-Raverot B, Puijalon S (2012) Nutrient enrichment affects the mechanical resistance of aquatic plants. Journal of Experimental Botany 63(17):6115-6123

McNickle GG, Cahill JF, Jr. (2009) Plant root growth and the marginal value theorem. Proceedings of the National Academy of Sciences 106(12): 4747–4751

Mitsch WJ, Gosselink JG (2000) Wetlands, Third Edition. John Wiley and Sons, Inc. New York, NY 920 pp.

Morris JT, Bradley PM (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. Limnology and Oceanography 44(3):699–702

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago, IL 608 pp.

Niklas KJ, Spatz HC (2012) Plant Physics. University of Chicago Press, Chicago, IL 426 pp.

Ponnamperuma FN (1972) The chemistry of submerged soils *In*: NC Brady (ed.) Advances in Agronomy, Volume 24. Academic Press, Inc. New York, NY 320 pp.

Ponnamperuma FN (1984) Effects of flooding on soils *In*: TT Kozlowski (ed.) Flooding and Plant Growth. Academic Press, Inc. New York, NY 345 pp.

Puijalon S, Bornette G (2004) Morphological variation of two taxonomically distant plant species along a natural flow velocity gradient. New Phytologist 163(3):651-660

Puijalon S, Bornette G, Sagnes P (2005) Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. Journal of Experimental Botany 56(412):777–786

Puijalon S, Bornette G (2006) Phenotypic plasticity and mechanical stress: Biomass partitioning and clonal growth of an aquatic plant species. American Journal of Botany 93(8):1090–1099

Puijalon S, Lena JP, Bornette G (2007) Interactive effects of nutrient and mechanical stresses on plant morphology. Annuals of Botany 100:1297–1305

Puijalon S, Piola F, Bornette G (2008) Abiotic stresses increase plant regeneration ability. Evolutionary Ecology 22:493–506

Puijalon S, Bouma TJ, Douady CJ, van Groenendael J, Anten NPR, Martel E, Bornette G (2011) Plant resistance to mechanical stress: evidence of an avoidance–tolerance trade-off. New Phytologist 191:1141–1149

Reddy KR, Delaune RD (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, New York, NY 774 pp.

Reish DJ, Geesey GG, Kauwling TJ, Wilkes FG, Mearns AJ, Oshida PS, Rossi SS (1980) Marine and estuarine pollution. Journal of the Water Pollution Control Federation 52(6):1980:1533-1575

Sculthorpe CC (1967) The Biology of Aquatic Vascular Plants. Edward Arnold, London, UK 610 pp.

Seago JL, Jr. Marsh LC, Stevens KJ, Soukup A, Votrubová O, Enstone DE (2005) A re-examination of the root cortex in wetland flowering plants with respect to aerenchyma. Annals of Botany 96:565–579

Soukup A, Votrubová O, Čížková H (2002) Development of anatomical structure of roots of *Phragmites australis*. The New Phytologist 153(2):277-287

Striker GG, Insausti P, Grimoldi AA, Vega AS (2007) Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. Plant, Cell and Environment 30:580–589

Striker GG (2012) Gustavo Gabriel Striker (2012). Flooding Stress on Plants: Anatomical, Morphological and Physiological Responses, *In*: J Mworia (Ed.) Botany. InTech Europe Rijeka, Croatia pp. 1-28

Sundareshwar PV, Morris JT, Koepfler EK, Fornwalt B (2003) Phosphorus limitation of coastal ecosystem processes. Science 299:563-565

Swarzenski CM, Doyle TW, Fry B, Hargis TG (2008) Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. Biogeochemistry 90:49–63

Turner RE, Howes BL, Teal JM, Milan CS, Swenson EM, Goehringer-Toner DD (2009) Salt marshes and eutrophication: An unsustainable outcome. Limnology and Oceanography 54(5):1634–1642

Turner RE (2011) Beneath the salt marsh canopy: Loss of soil strength with increasing nutrient loads. Estuaries and Coasts 33:1084-1093. doi: 10.1007/s12237-010-9341-y

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: Limnology and Oceanography (21)2:245-252

Visser EJW, Sandy ER (2009) The effects of flooding on four common Louisiana marsh plants. Gulf of Mexico Science 1:21–29

Welch HL, Coupe RH, Aulenbach BT (2014) Concentrations and transport of suspended sediment, nutrients, and pesticides in the Lower Mississippi-Atchafalaya River subbasin during the 2011 Mississippi River flood, April through July. US Geological Survey Scientific Investigations Report 2014–5100, 44 pp. http://dx.doi/10.3133/sir20145100

Wigand C, Brennan P, Stolt M, Holt M, Ryba S (2009) Soil respiration rates in coastal marshes subject to increasing watershed nitrogen loads in southern New England, USA. Wetlands 29(3):952–963

Willey N (2016) Environmental Plant Physiology. Garland Science, Taylor and Francis Group, LLC New York, NY 390 pp.

CHAPTER 6
THE TENSILE ROOT STRENGTH OF *SPARTINA PATENS* DECLINES WITH EXPOSURE TO MULTIPLE STRESSORS

INTRODUCTION

Wetlands are usually receiving basins for a plethora of anthropogenic xenobiotics due to their hydrogeomorphic position in the landscape. In addition, a host of human activities take place in close proximity to wetlands. These activities include agricultural operations, forestry, urban/suburban development, resource extraction, and onshore and marine transportation. These activities may produce drastic changes in the landscape and generate a number of chemical compounds that may stress terrestrial, aquatic, and wetland ecosystems.

Coastal wetland ecosystems are threatened because of dense human populations in their midst. Crowell et al. (2010) reported that in 2010, 39% of the population of the United States lived in counties directly adjacent to the coast. The human population can generate an influx of numerous pollutants into coastal environments such as petroleum by-products, human personal care products, excessive sediment loads, high nutrient loads, and pesticides. As a result, wetland ecosystems may be subjected to multiple stressors that disrupt or compromise vital ecosystem functions and services. For example, extensive anthropogenic habitat destruction and alteration of the landscape have modified natural hydrologic regimes.

Flood control efforts such as channelization of streams, the construction of dams and levees, and flow diversion projects have disrupted the natural hydropattern of wetlands, the result being excessive inundation and extended residence times of floodwaters within wetland habitats (Mitsch and Gosselink 2000, Jackson 2006, Keddy 2010, Willey 2016). Flooding induces oxygen stress on wetland plants because inundated soils severely curtail gas transport and exchange between plants and the atmosphere. In addition, saturated soils produce lower

oxidation-reduction potentials (hereafter, redox potential), which can facilitate the accumulation of compounds that are toxic to plants such as soluble Fe^{2+} and Mn^{2+}, sulfides, ethanol, acetaldehyde, acetic acid, lactic acid and formic acid (Kozlowski 1984, Armstrong et al. 1994, Cronk and Fennessy 2001, Evans 2003, Fieldler et al. 2007, Reddy and Delaune 2008, Striker 2012). Flood-induced stress can also inhibit photosynthesis and reduce carbon fixation within the plant (Justin and Armstrong 1987, Colmer and Voesenek 2009,)

Photosynthesis may also be inhibited by the action of herbicides such as atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine), which targets the transfer of electrons to Photosystem II (Solomon et al. 1996, Krieger-Liszkay and Rutherford 1998, Fufezan et al. 2002, Ghosh and Philip 2006, USEPA 2016) . The interruption of electron transfer during this phase of photosynthesis prevents the plant from synthesizing adenosine triphosphate (ATP) for energy replenishment. However, the lethal mode of action of atrazine is the result of oxidative stress rather than starvation (Zhu et al. 2009). The blockage of electron transfer during photosynthesis induces a rapid and prolonged accumulation of reactive oxygen species (ROS), such as superoxide, peroxide, and a hydroxyl radical, which can oxidize plant tissue (Dat et al. 2000, Sharma et al. 2012).

For example, nutrient loading from nonpoint pollution with has been implicated in the loss of coastal wetlands. Many researchers have demonstrated that excess nutrient influxes to coastal wetlands have led to higher rates of soil respiration (Morris and Bradley 1999, Wigand et al. 2009), a reduction in belowground biomass (Valiela et al. 1976, Morris and Bradley 1999, Darby and Turner 2008a, Deegan et al. 2012, Graham and Mendelssohn 2014, Graham and Mendelssohn 2016) and lower soil strength (Darby and Turner 2008b, Swarzenski et al. 2008,

Turner et al. 2009, Turner 2011). As a result, eutrophic conditions, in concert with flood adaptations that reduce root biomass, may compromise tensile root strength.

Multiple stressors that weaken the belowground biomass of wetland plants may reduce the resistance of the vegetation community to biomechanical forces that can erode the resilience of the ecosystem. For example, Naidoo et al. (1992) found increased alcohol dehydrogenase activity in *Spartina patens* in salinity treatments under hypoxic conditions, an indication of inadequate aerenchyma development to support aerobic root respiration. The decrease in gas exchange could induce a shift to anaerobic respiration, which would increase the carbon demand and weaken the structural integrity of the roots, thereby increasing the probability of plant loss to erosion. In addition, rising sea levels and the increased frequency of tropical cyclones due to climate change may increase the physiological and biomechanical stress on coastal macrophytes by inducing changes in salinity levels, prolonged inundation, and the impact of hydrologic forces on plants from storm surges. The uprooting and loss of coastal wetland plants can accelerate the erosion of coastal wetlands and force a regime shift into an open-water estuarine habitat that will result in the collapse of the wetland ecosystem. Consequently, coastal wetlands that are subjected to multiple stressors may undergo changes in plant communities, increased erosion, altered biogeochemical cycles, and diminished the ecosystem services and functions that help sustain human communities. Therefore, the determination of the tensile root strength of a dominant coastal macrophyte such as *Spartina patens* may be a method to measure the resistance of the coastal plant community to erosive forces and ascertain the resilience of coastal wetland ecosystems.

The objective of this study was to investigate the effects of flood duration and different combinations of nutrient addition and atrazine on the tensile root strength of the wetland

macrophyte *S. patens*. The study tested the hypotheses that flood duration, atrazine exposure, and nutrient addition have synergistic effects on the belowground biomass of *S. patens* that reduce its tensile root strength.

MATERIALS AND METHODS

Atrazine-Flood Duration-Nutrient Interaction Experiment

Plants were grown under natural light conditions in the greenhouses of Louisiana State University at Baton Rouge, LA, USA. The experimental design consisted of a 3 x 2 x 2 x 4 factorial design with atrazine, nutrient addition, and flood duration as the main effects. *Spartina patens* plugs were obtained from the Green Seasons Nursery in Tampa, FL. The samples were transplanted to 9.45-liter (2.5-gallon) plastic pots filled with 5.5 L of a mixture of 65% sphagnum peat (Premier Sphagnum Peat Moss; 100% Canadian peat moss, no added fertilizer or nutrients), 30% clay/silt mixture, and 5% sand. The sand, silt, and clay components were obtained by LSU greenhouse staff from the Sterlington soil series (coarse-silty, mixed thermic Typic Hapludalfs) in the Mississippi River floodplain in West Baton Rouge Parish, LA. The soil texture of clay/silt components was estimated by a texture-by-feel field technique and determined to be sandy clay loam (Brady and Weil 2002). During the experiment, the treatments were rotated monthly on a reverse-orientation basis (e.g. From south to north, and west to east) to reduce the variation in environmental conditions.

The nitrogen and phosphorus nutrient treatments consisted of granular reagent grade calcium nitrate tetrahydrate [$Ca(NO_3)_2 \bullet 4H_2O$] and granular laboratory grade potassium phosphate [K_3PO_4] (Fisher Scientific; Nazareth, PA). Nutrient treatments, added monthly, were as follows: High Nitrogen (HN, 5.0 mg L^{-1}), Low Nitrogen (LN, 1.75 mg L^{-1}), High Phosphorus (HP, 0.30 mg L^{-1}), Low Phosphorus (LP, 0.10 mg L^{-1}), High Nitrogen x Low Phosphorus (Np),

and Low Nitrogen x High Phosphorus (nP). A 25 ppm atrazine stock solution was formed by placing Pestanal® Sigma-ALDRICH atrazine in deionized water (Starr et al. 2017). Because atrazine has a moderate solubility in water (30 ppm at 20 °C), the solution was placed on a hot plate with a magnetic stirrer, heated at 23 °C, and mixed with magnetic stirring rods for a 24 hour period before the experiment to ensure the atrazine was fully dissolved (Starr et al. 2017). Atrazine treatments, which were also added monthly, were as follows: High (5.0 micrograms per liter [$\mu g\ L^{-1}$]), Medium (3.0 $\mu g\ L^{-1}$), and Low (1.0 $\mu g\ L^{-1}$).

The flood duration experimental unit set-up consisted of placing each 9.45-L plastic pot inside an 18.9-L (5-gallon) high-density plastic bucket and filling the bucket with deionized water to 15 cm above the soil surface in the plastic pot. The flood duration treatments were 50% of the designated time frames: Bi-Weekly (14 days: 7 days flooded, 7 days saturated) and Monthly (30 days: 15 days flooded, 15 days saturated). Water levels were manipulated by placing bricks underneath the plastic pots during the drained period and removing the bricks during the flood period. During the drained phase, water levels were maintained ~1.75 cm above the soil surface to ensure saturated soil conditions. Soil temperature, pH, and redox potential were measured monthly, prior to the addition of nutrient and atrazine treatments (for details see Hollis and Turner 2018). Soil temperature was measured by a soil probe thermometer to the nearest 0.1 °C. The pH of the soil pore water was obtained with a Lisle vacuum pump (Lisle Corporation, Clarinda, IA) and measured with a Hach HQ 40d multi-parameter meter (Hach Industries Loveland, CO). Redox potential was measured with 45 cm-long standard platinum probes with after Reddy and Delaune (2008) and a Corning calomel reference probe (Corning, Inc. Corning, NY) that were connected to a Fluke 73 Multimeter (John Fluke Manufacturing, Everett WA). A correction of +244 mV was added to redox measurements to compensate for the

difference in redox potential between the calomel probe and standard hydrogen reference electrode (Reddy and Delaune 2008). The experiment was conducted for a total of 122 days from 1 May 2016 until 31 August 2016.

Tensile Strength Testing

Tensile strength testing was conducted only on live roots. The Small size class (0.5–1.0 mm) was selected for testing because of the high numbers of roots within this diameter range and the increased probability of conducting successful tensile strength tests. A mean of six tests were conducted for every successful tensile strength test. A successful test consisted of root samples that failed between the supports of the test stand, whereas roots that failed at the supports were considered unsuccessful tests. Live roots and rhizomes were differentiated from dead roots by their white, turgid, and translucent appearance while dead roots are dark and flaccid (Darby and Turner 2008a). However, many live roots were stained by soil deposits and they were separated from dead roots by the presence of turgor, bifurcations of fine roots, and their ability to float. Three individual root metrics were measured: mass, length, and diameter. Cross-sectional area (mm^2) and volume (mm^3) were calculated from length and diameter measurements after tensile strength testing was performed. Root length was measured to the nearest 0.1 mm with a Scale Master© Classic digital planimeter (Calculated Industries, Carson, NV USA). The mean root diameter was measured to the nearest 0.1 mm with a Starrett digital IP67 micrometer. The measurements were taken at both ends and at the middle of each root and averaged. Root samples were weighed to the nearest 0.1 milligram (mg) to estimate individual mass. A Mecmesin MultiTest 1–d motorized stand (Mecmesin Limited; Sinfold, West Sussex, UK) was used to test tensile root strength in Newtons (N). Individual roots were secured to two support clamps that were perpendicular to the base of the test stand. The contact surfaces of the clamps

provided 1.25 x 2.50 cm of area and were lined with fine sandpaper to reduce or eliminate slippage. The test stand was activated and the top support was pulled upward by a vertical hydraulic piston until the root exhibited structural failure. The load that induced failure at that point, or breaking force, was recorded as tensile strength.

Tissue Sample Testing

Samples of live leaf and root tissue of each experimental unit and the control were collected at the end of the experiment and after tensile strength testing and sent to the LSU Soil Testing and Plant Analysis Laboratory to determine the carbon, nitrogen, and phosphorus tissue content testing. The results of these tests were used to calculate carbon-nitrogen (C:N) and nitrogen-phosphorus ratios molar ratios (mM g^{-1}).

Statistical Analyses

I conducted an analysis of variance (ANOVA) using JMP v. 13 software (SAS Cary, NC) to test for differences in the mean tensile strength of roots by the atrazine, nutrient, and flood duration main effects. Significant differences between the tensile root strength means were determined using a Tukey-Kramer Honest Significant Difference (HSD) test. I tested for interactive effects among the main effects by segregating the tensile strength data of one main effect into subsets and conducting one-way ANOVA of tensile root strength for each level of the other main effect. The data are reported as the mean ± 1 standard error of the mean unless otherwise noted. Homoscedasticity and normality of residuals were determined with Brown-Forsythe and Shapiro-Wilk tests, respectively. Data that did not meet the assumptions of ANOVA were tested with a Welch's ANOVA, and differences between the tensile strength means were determined using a Steel-Dwass nonparametric multiple comparison test. Interactive effects of treatment combinations were determined by using a Kolmogorov-Smirnov goodness-

of-fit test to compare the data distribution of the combination with that of the strongest main effect of the treatment combination. Statistical significance between the soil temperature, redox potential, and pH parameter data were tested using a Student's *t*-test. The differences among the nutrient and the Carbon:Nitrogen:Phosphorus (CNP) ratios were tested with a one-way ANOVA. All statistical tests were performed at a significance level of $p < 0.05$.

RESULTS

Tensile Root Strength

A one-way Welch's ANOVA with nutrient addition as the main effect revealed significant difference in tensile root strength between the two levels of treatment and Control (Fig. 6.1, 4.10 ± 0.18 N, $F = 49.7$, $p < 0.0001$). There was no significant difference in tensile root strength between the High Nitrogen-High Phosphorus (NP) (1.74 ± 0.18 Newtons, [N]) and the Low Nitrogen-Low Phosphorus (np) (1.67 ± 0.18 N) treatments and the grand tensile root strength mean was 2.50 ± 0.15 N. Likewise, a one-way Welch's ANOVA of tensile root strength in the High atrazine data subset found no significant difference between the two nutrient treatments; however, the Control tensile root strength (4.10 ± 0.15 N) was twice that of the NP (1.80 ± 0.11 N) and np (1.63 ± 0.12 N) treatments and the grand tensile strength mean was 2.20 ± 0.16 N (Fig. 6.2a, $F = 56.9$, $p < 0.0001$).

In the Medium atrazine data subset, a one-way Welch's ANOVA of tensile root strength also found that the Control tensile root strength (4.10 ± 0.16 N) was twice that of the NP (1.65 ± 0.11 N) and np (1.93 ± 0.11 N) treatments (Fig. 6.2b, $F = 56.7$, $p < 0.0001$). There was no significant difference between the NP and np treatments. The tensile root strength grand mean was 2.25 ± 0.16 N.

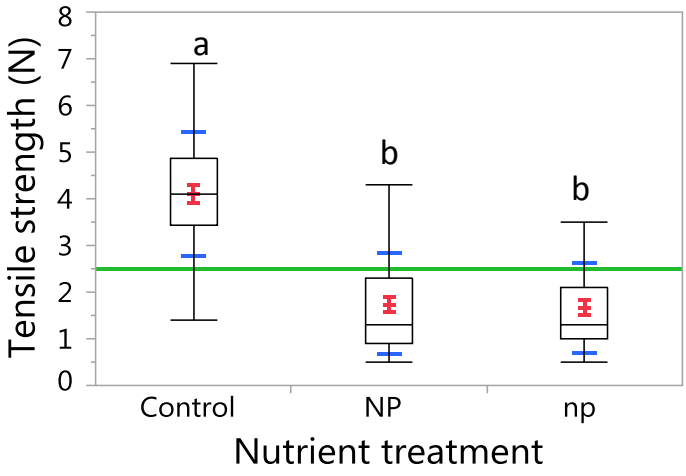


Fig. 6.1 One-way Welch's ANOVA of tensile root strength with nutrient addition as the main effect for the atrazine-flood duration-nutrient interaction greenhouse experiment. There were significant differences between control and nutrient treatments ($p < 0.0001$). The box plot whiskers represent the maximum and minimum values. The blue horizontal lines denote ± 1 standard deviation. The center horizontal red line represents the group mean and the two red lines above and below represents ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean

A one-way Welch's ANOVA of tensile root strength in the Low atrazine data subset revealed significant differences in tensile root strength between the two nutrient treatments and control (Fig. 6.2c, $F = 68.2$, $p < 0.0001$). The Control (4.10 ± 0.17 N) was two times stronger than both the NP (2.04 ± 0.12 N) and the np (1.84 ± 0.12 N) treatments. However, there was no significant difference in tensile root strength between the two nutrient treatments and the grand tensile root strength mean was 2.37 ± 0.17 N.

In the Monthly flood duration data subset, the Control (4.10 ± 0.16 N) was two times stronger than the NP (1.86 ± 0.09 N) and np (1.81 ± 0.09 N) treatments. A one-way Welch's ANOVA of tensile root strength revealed significant differences in tensile root strength between the two nutrient treatments and Control (Fig. 6.3a, $F = 53.6$, $p < 0.0001$). However, there was no

significant difference between the two nutrient treatments and the tensile root strength grand mean was 2.16 ± 0.15 N.

In the Bi-Weekly flood duration data subset, a one-way Welch's ANOVA of tensile root strength found that the Control (4.10 ± 0.16 N) was two times stronger than the NP (1.78 ± 0.09 N) and np (1.82 ± 0.09 N) treatments (Fig. 6.3b, $F = 54.7$, $p < 0.0001$). There was no significant difference in tensile root strength between the NP and np treatments and the tensile root strength grand mean was 2.13 ± 0.16 N.

A one-way Welch's ANOVA with flood duration as the main effect revealed significant differences in tensile root strength between the two levels of flood duration and Control (Fig. 6.4, $F = 41.8$, $p < 0.0001$). There was no significant difference in tensile root strength between the Bi-Weekly (2.05 ± 0.17 N) and the Monthly (1.86 ± 0.17 N) treatments and the grand tensile root strength mean was 2.67 ± 0.15 N.

In the High Nitrogen-High Phosphorus nutrient addition data subset, a one-way Welch's ANOVA of tensile root strength found that the Control (4.10 ± 0.16 N) was two times stronger than the Bi-Weekly (1.78 ± 0.09 N) and Monthly (1.86 ± 0.09 N) flood duration treatments (Fig. 6.5a, $F = 54.0$, $p < 0.0001$). There was no significant difference in tensile root strength between the Bi-Weekly and Monthly treatments and the tensile root strength grand mean was 2.15 ± 0.16 N. A one-way Welch's ANOVA of tensile root strength in the Low Nitrogen-Low Phosphorus data subset yielded significant differences in tensile root strength between the two flood duration treatments and control (Fig. 6.5b, $F = 54.2$, $p < 0.0001$). The Control (4.10 ± 0.16 N) was two times stronger than both the Bi-Weekly (1.82 ± 0.09 N) and the Monthly (1.81 ± 0.09 N) treatments. However, there was no significant difference in tensile root strength between the two flood duration treatments and the grand tensile root strength mean was 2.14 ± 0.16 N.

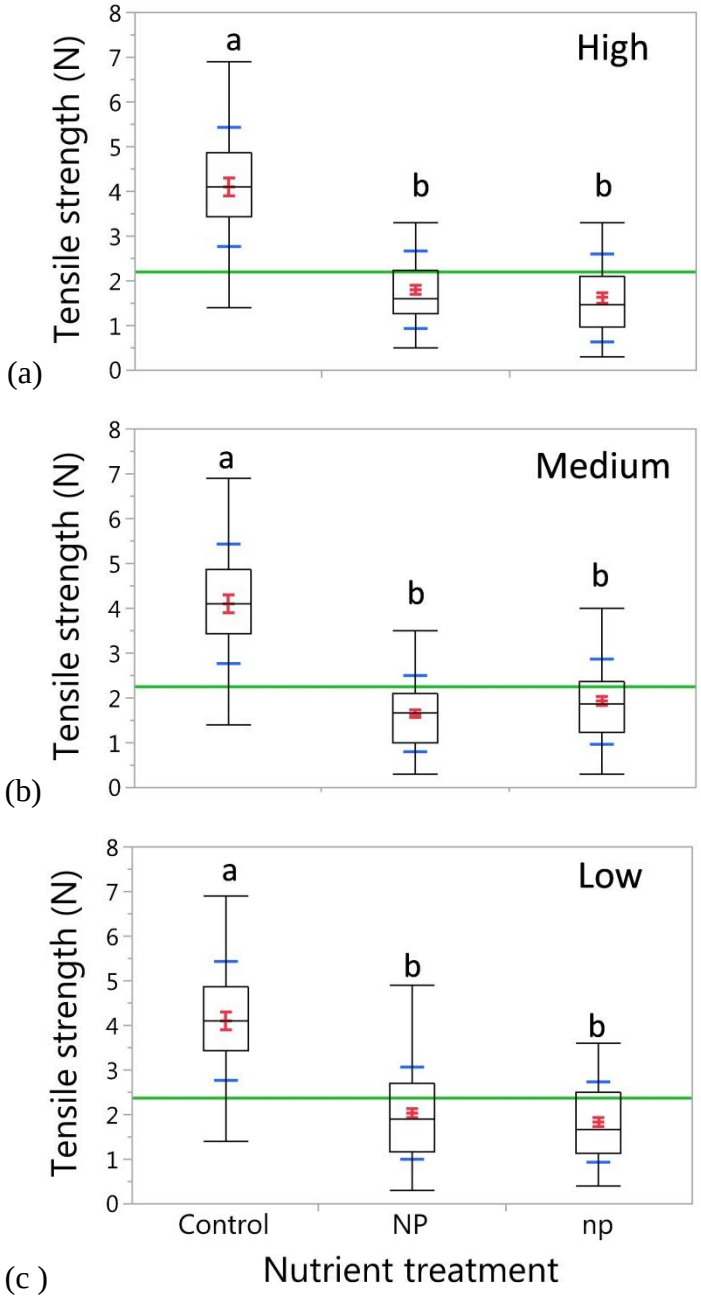
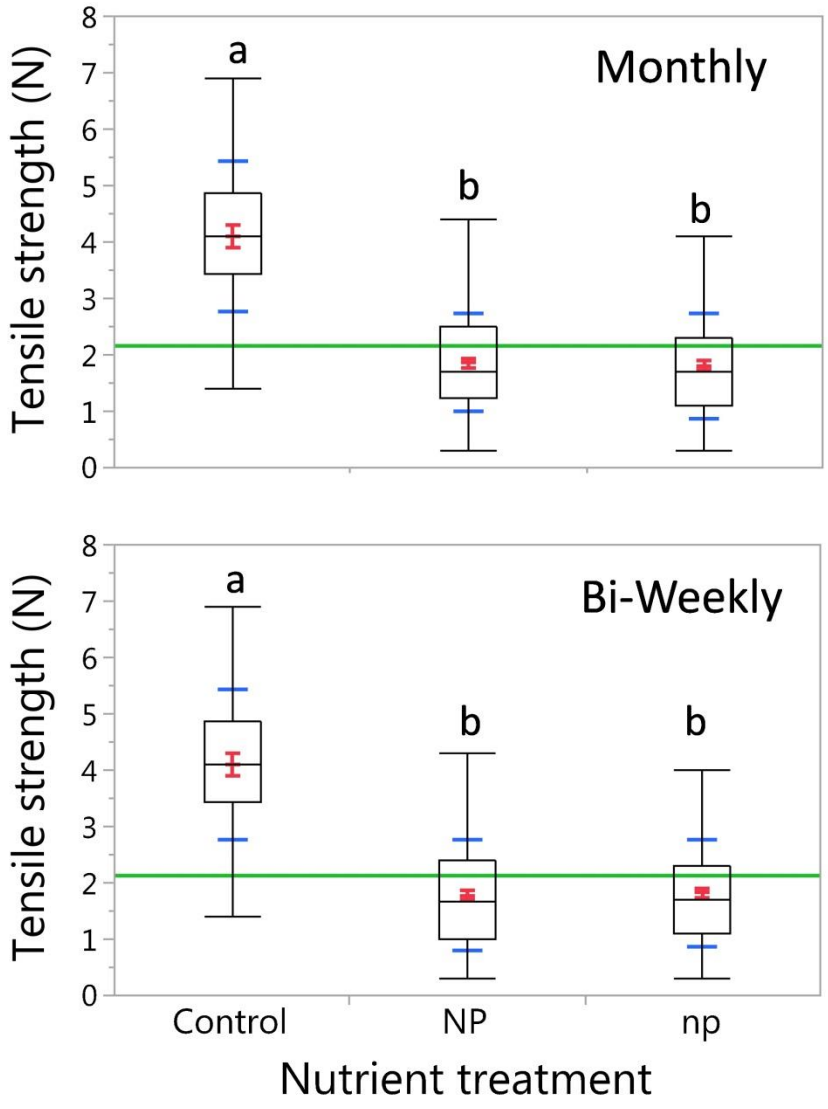


Fig. 6.2 Box and whisker plots of a one-way Welch's ANOVA of tensile root strength for the (a) High Atrazine (b) Medium Atrazine, and (c) Low Atrazine treatment data subsets to test for interactive effects between nutrient and atrazine treatments. There were significant differences between control and nutrient treatments (Table 6.1, $p < 0.0001$) in all subsets. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments



(a)

(b)

Fig. 6.3 Box and whisker plots of one-way Welch's ANOVA of tensile root strength with nutrient addition as the main effect for the (a) Monthly, and (b) Bi-Weekly flood duration treatment data subsets to test for interactive effects between nutrient and flood duration treatments. There were significant differences between control and nutrient treatments ($p <$ 0.0001) in both subsets. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

A one-way Welch's ANOVA of tensile root strength in the High atrazine data subset revealed significant differences in tensile root strength between the two flood duration treatments and Control (Fig. 6.6a, $F = 56.7$, $p < 0.0001$).

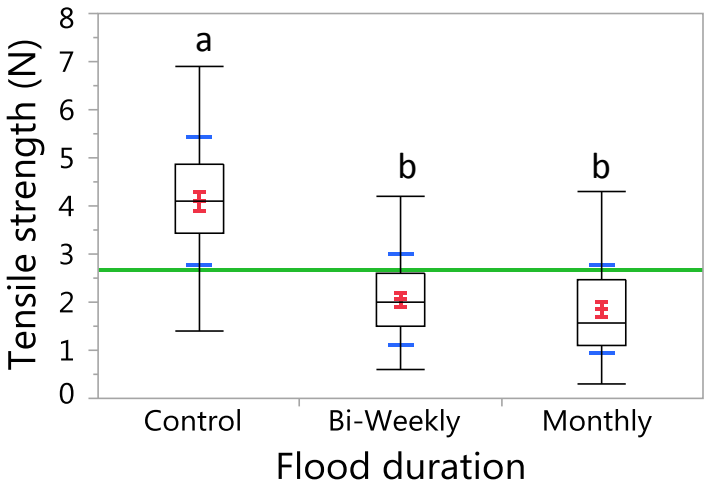


Fig. 6.4 Box and whisker plots of one-way Welch's ANOVA of tensile root strength with flood duration as the main effect for the atrazine-flood duration-nutrient interaction greenhouse experiment. There were significant differences between control and flood duration treatments (Table 6.1, $p < 0.0001$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

The Control (4.10 ± 0.16 N) was two times stronger than both the Bi-Weekly (1.72± 0.11N) and the Monthly (1.73 ± 0.11 N) treatments. There was no significant difference in tensile root strength between the two nutrient treatments and the grand tensile root strength mean was 2.20 ± 0.16 N.

Similarly, the Control (4.10 ± 0.16 N) was two times stronger than the Bi-Weekly (1.70 ± 0.11 N) and Monthly (1.88 ± 0.11 N) treatments in the Medium atrazine data subset. A one-way Welch's ANOVA of tensile root strength revealed significant differences in tensile root strength between the two flood duration treatments and Control (Fig. 6.6b, $F = 54.3$, $p < 0.0001$). However, there was no significant difference between the two flood duration treatments and the tensile root strength grand mean was 2.25 ± 0.16 N.

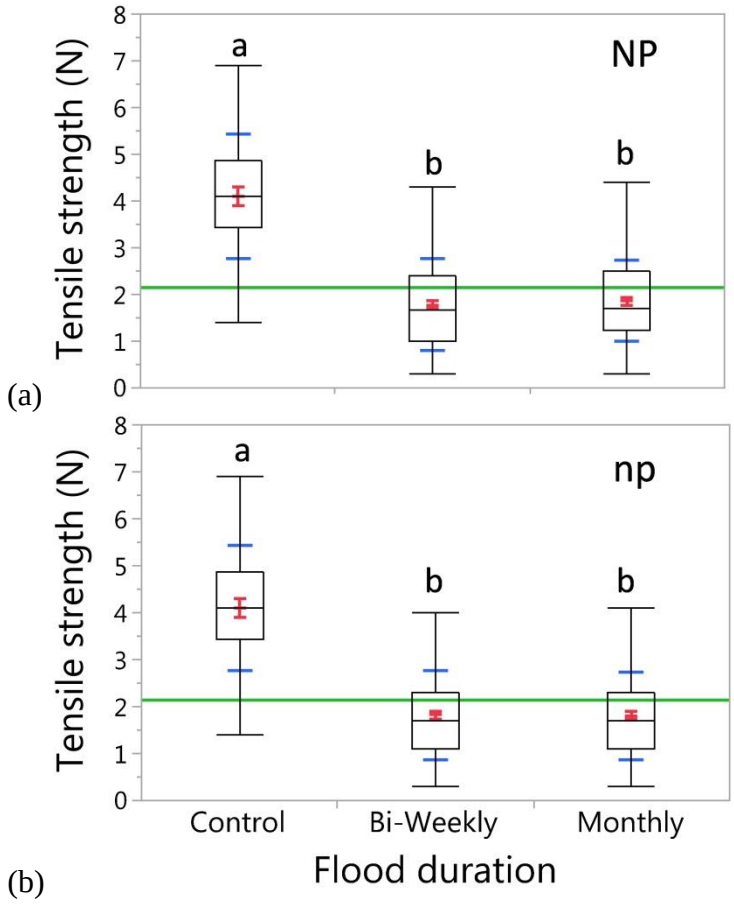


Fig. 6.5 Box and whisker plots of one-way Welch's ANOVA of tensile root strength with flood duration as the main effect for the (a) High Nitrogen-High Phosphorus (NP) and (b) Low Nitrogen-Low Phosphorus (np) nutrient addition subsets to test for interactive effects between nutrient and flood duration treatments. There were significant differences between control and flood duration treatments (Table 6.1, $p < 0.0001$) in both subsets. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

In the Low atrazine data subset, a one-way Welch's ANOVA of tensile root strength found that the Control (4.10 ± 0.16 N) was two times stronger than the Bi-Weekly (1.98 ± 0.11 N) and Monthly (1.89 ± 0.11 N) flood duration treatments (Fig. 6.6c, $F = 46.7$, $p < 0.0001$). There was no significant difference in tensile root strength between the Bi-Weekly and Monthly units and the tensile root strength grand mean was 2.37 ± 0.16 N.

Table 6.1 Summary statistics of the tensile root strength response variable for the nutrient, atrazine, and flood duration main effects and main effect subset (in parentheses) testing for interactive effects. Statistical significance is indicated by *p*-values < 0.05

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Nutrient	120	n/a	n/a	n/a	1.71	2.50	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.18	1.33	n/a
High Nitrogen-Phosphorus (NP)	40	4.9	0.5	1.74	n/a	n/a	0.18	1.09	< 0.0001
Low Nitrogen-Phosphorus (np)	40	5.8	0.5	1.67	n/a	n/a	0.18	0.98	< 0.0001
Nutrient (Bi-Weekly)	120	n/a	n/a	n/a	1.80	2.13	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
High Nitrogen-Phosphorus (NP)	40	4.9	0.3	1.78	n/a	n/a	0.09	0.98	< 0.0001
Low Nitrogen-Phosphorus (np)	40	4.7	0.3	1.82	n/a	n/a	0.09	0.95	< 0.0001
Nutrient (Monthly)	120	n/a	n/a	n/a	1.83	2.16	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.15	1.33	n/a
High Nitrogen-Phosphorus (NP)	40	4.4	0.3	1.86	n/a	n/a	0.09	0.88	< 0.0001
Low Nitrogen-Phosphorus (np)	40	5.8	0.3	1.81	n/a	n/a	0.09	0.93	< 0.0001
Nutrient (Low)	120	n/a	n/a	n/a	1.94	2.37	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.17	1.33	n/a
High Nitrogen-Phosphorus (NP)	40	4.9	0.3	2.04	n/a	n/a	0.12	1.03	< 0.0001
Low Nitrogen-Phosphorus (np)	40	4.7	0.4	1.84	n/a	n/a	0.12	0.90	< 0.0001
Nutrient (Medium)	120	n/a	n/a	n/a	1.79	2.25	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
High Nitrogen-Phosphorus (NP)	40	4.4	0.3	1.65	n/a	n/a	0.11	0.84	< 0.0001
Low Nitrogen-Phosphorus (np)	40	4.7	0.3	1.93	n/a	n/a	0.11	0.95	< 0.0001

(Table 6.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Nutrient (High)	120	n/a	n/a	n/a	1.81	2.20	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
High Nitrogen-Phosphorus (NP)	40	4.6	0.5	1.80	n/a	n/a	0.11	0.85	< 0.0001
Low Nitrogen-Phosphorus (np)	40	5.8	0.3	1.63	n/a	n/a	0.12	0.98	< 0.0001
Flood Duration	120	n/a	n/a	n/a	1.96	2.67	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.17	1.33	n/a
Bi-Weekly	40	4.6	0.6	2.05	n/a	n/a	0.17	0.95	< 0.0001
Monthly	40	4.3	0.3	1.86	n/a	n/a	0.17	0.93	< 0.0001
Flood Duration (NP)	120	n/a	n/a	n/a	1.82	2.15	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Weekly	40	4.9	0.3	1.78	n/a	n/a	0.09	0.98	< 0.0001
Bi-Monthly	40	4.4	0.3	1.86	n/a	n/a	0.09	0.88	< 0.0001
Flood Duration (np)	120	n/a	n/a	n/a	1.81	2.14	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Bi-Weekly	40	4.7	0.3	1.82	n/a	n/a	0.09	0.95	< 0.0001
Monthly	40	5.8	0.3	1.81	n/a	n/a	0.09	0.93	< 0.0001

(Table 6.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Flood Duration (Low)	120	n/a	n/a	n/a	1.94	2.37	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.17	1.33	n/a
Bi-Weekly	40	4.9	0.5	1.99	n/a	n/a	0.12	1.02	< 0.0001
Monthly	40	4.2	0.3	1.89	n/a	n/a	0.12	0.92	< 0.0001
Flood Duration (Medium)	120	n/a	n/a	n/a	1.79	2.25	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Bi-Weekly	40	4.3	0.3	1.70	n/a	n/a	0.11	0.95	< 0.0001
Monthly	40	4.7	0.3	1.88	n/a	n/a	0.11	0.86	< 0.0001
Flood Duration (High)	120	n/a	n/a	n/a	1.72	2.20	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Bi-Weekly	40	4.6	0.3	1.72	n/a	n/a	0.11	0.91	< 0.0001
Monthly	40	5.8	0.3	1.73	n/a	n/a	0.11	0.92	< 0.0001
Atrazine	160	n/a	n/a	n/a	1.92	2.46	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Low	40	4.9	0.4	2.06	n/a	n/a	0.16	1.05	< 0.0001
Medium	40	4.4	0.5	1.99	n/a	n/a	0.16	0.91	< 0.0001
High	40	4.6	0.6	1.71	n/a	n/a	0.16	0.86	< 0.0001

(Table 6.1 continued.)

Source	n	Max	Min	Mean	Group Mean	Grand Mean	SE	SD	*p*-value
Atrazine (NP)	160	n/a	n/a	n/a	1.91	2.15	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Low	40	4.9	0.4	2.04	n/a	n/a	0.11	1.03	< 0.0001
Medium	40	4.4	0.3	1.65	n/a	n/a	0.11	0.84	< 0.0001
High	40	4.6	0.5	1.78	n/a	n/a	0.11	0.88	< 0.0001
Atrazine (np)	160	n/a	n/a	n/a	1.79	2.14	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Low	40	4.7	0.4	1.84	n/a	n/a	0.11	0.90	< 0.0001
Medium	40	4.7	0.3	1.93	n/a	n/a	0.11	0.95	< 0.0001
High	40	5.8	0.3	1.67	n/a	n/a	0.11	0.95	< 0.0001
Atrazine (Bi-Weekly)	160	n/a	n/a	n/a	1.84	2.13	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.16	1.33	n/a
Low	40	4.9	0.5	1.99	n/a	n/a	0.11	1.02	< 0.0001
Medium	40	4.3	0.3	1.70	n/a	n/a	0.11	0.95	< 0.0001
High	40	4.6	0.3	1.72	n/a	n/a	0.11	0.91	< 0.0001
Atrazine (Monthly)	160	n/a	n/a	n/a	1.81	2.16	n/a	n/a	< 0.0001
Control	40	7.1	1.4	4.10	n/a	n/a	0.15	1.33	n/a
Low	40	4.2	0.3	1.89	n/a	n/a	0.11	0.92	< 0.0001
Medium	40	4.7	0.3	1.88	n/a	n/a	0.11	0.86	< 0.0001
High	40	5.8	0.3	1.73	n/a	n/a	0.11	0.92	< 0.0001

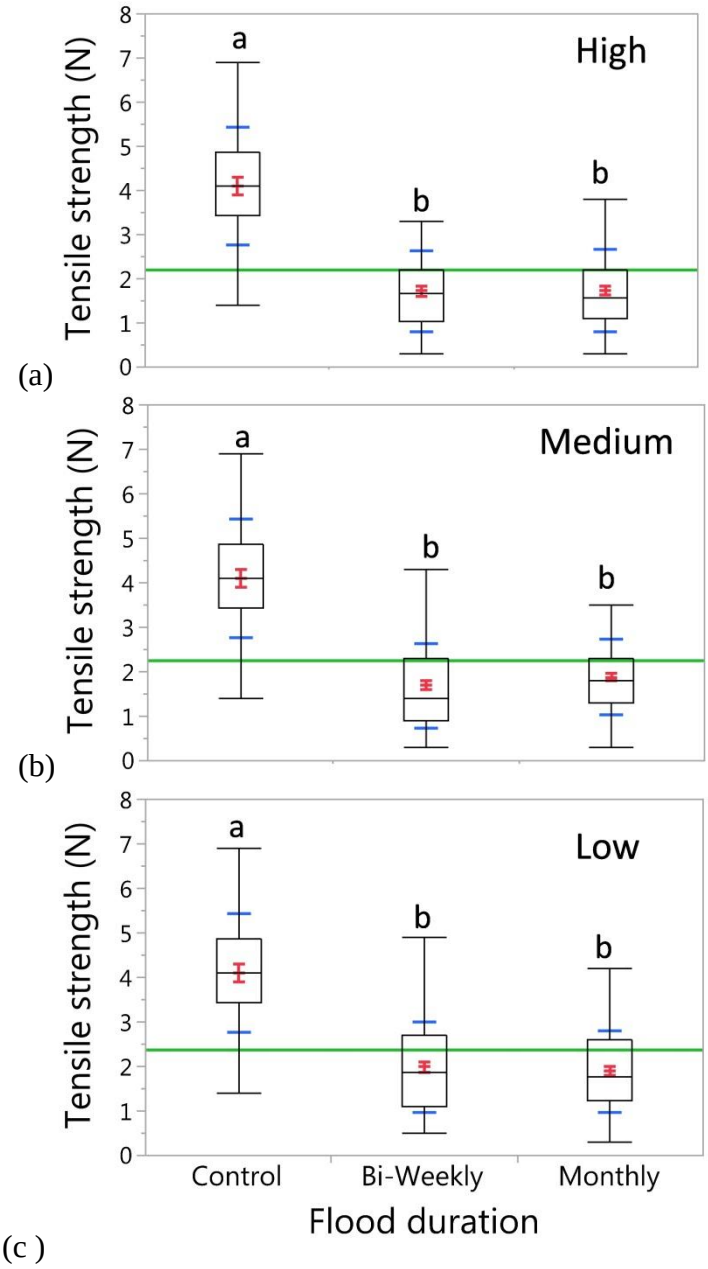


Fig. 6.6 Box and whisker plots of One-way Welch's ANOVA of tensile root strength with flood duration as the main effect for the (a) High Atrazine (b) Medium Atrazine, and (c) Low Atrazine data subsets to test for interactive effects between atrazine and flood duration treatments. There were significant differences between control and flood duration treatments (Table 6.1, $p < 0.0001$) in all subsets. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

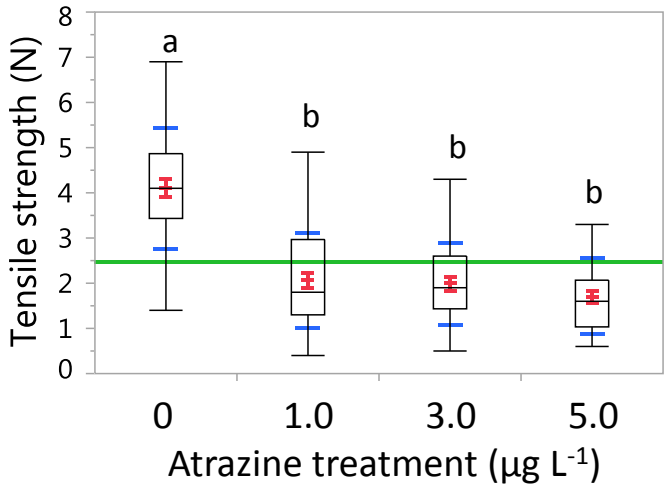


Fig. 6.7 Box and whisker plot of one-way Welch's ANOVA of tensile root strength with atrazine as the main effect for the atrazine-flood duration-nutrient interaction greenhouse experiment. There were significant differences between control and flood duration treatments ($p < 0.0001$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

A one-way Welch's ANOVA with atrazine as the main effect revealed significant differences in tensile root strength between the three levels of atrazine and Control (Fig. 6.7, $F = 31.9$, $p < 0.0001$). The Control tensile root strength was two times stronger than the tensile root strength of all three atrazine treatments. There were no significant differences in tensile root strength between the High (1.71 ± 0.16 N), Medium (1.99 ± 0.16 N), and Low (2.06 ± 0.16 N) treatments and the grand tensile root strength mean was 2.46 ± 0.15 N.

In the High Nitrogen-High Phosphorus nutrient addition data subset, a one-way Welch's ANOVA of tensile root strength found that the Control (4.10 ± 0.16 N) was two times stronger than the High (1.78 ± 0.11 N), Medium (1.65 ± 0.11 N), and Low (2.03 ± 0.11 N) atrazine treatments (Fig. 6.8a, $F = 54.0$, $p < 0.0001$). There were no significant differences in tensile root strength among the three atrazine treatments and the tensile root strength grand mean was 2.15 ± 0.16 N.

Likewise, a one-way Welch's ANOVA of tensile root strength in the Low Nitrogen-Low Phosphorus nutrient addition data subset found significant differences between the three atrazine treatments and Control (Fig. 6.8b, $F = 37.1$, $p < 0.0001$). However, the Control (4.19 ± 0.15 N) was two times stronger than the High (1.67 ± 0.11 N), Medium (1.93 ± 0.11 N), and Low (1.84 ± 0.11 N) atrazine treatments. There were no significant differences in tensile root strength among the three atrazine treatments and the tensile root strength grand mean was 2.14 ± 0.16 N.

In the Monthly flood duration data subset, the Control (4.10 ± 0.15 N) was two times stronger than the High (1.73 ± 0.11 N), Medium (1.88 ± 0.11 N), and Low (1.89 ± 0.11 N) treatments. A one-way Welch's ANOVA of tensile root strength revealed significant differences in tensile root strength between the three atrazine treatments and Control (Fig. 6.9a, $F = 36.0$, $p < 0.0001$). However, there were no significant differences among the three atrazine treatments and the tensile root strength grand mean was 2.16 ± 0.15 N.

A one-way Welch's ANOVA of tensile root strength in the Bi-Weekly flood duration data subset with atrazine as the main effect revealed significant differences in tensile root strength between the three levels of atrazine and Control (Fig. 6.9b, $F = 38.2$, $p < 0.0001$). The Control tensile root strength was two times stronger than the tensile root strength of all three atrazine treatments. There were no significant differences in tensile root strength among the High (1.72 ± 0.11 N), Medium (1.70 ± 0.11 N), and Low (1.99 ± 0.11 N) treatments and the grand tensile root strength mean was 2.13 ± 0.16 N.

Soil Parameters

A Student's *t*-test revealed no significant difference tensile root strength among the soil temperatures or between the three atrazine treatments or control ($p > 0.05$). There were significant differences in soil pH between the two flood duration treatments and the Control

($p < 0.0001$). However, there was no significant difference between the two flood duration treatments in pH and no significant differences in the soil redox potential among the two flood duration treatments and Control (Table 6.3; $p < 0.0001$).

Table 6.2 Summary of one-way Welch's ANOVA tests of the tensile root strength response variable for the nutrient, flood duration, and atrazine main effects and main effect subset (in parentheses) testing for interactive effects. Statistical significance is indicated by *p*-values < 0.05

Source	[1]DFNum	[2]DFDen	F Ratio	*p*-value
Nutrient	2	76.9	49.7	< 0.0001
Nutrient (Bi-Weekly)	2	100.4	54.7	< 0.0001
Nutrient (Monthly)	2	98.9	53.5	< 0.0001
Nutrient (Low)	2	95.2	47.7	< 0.0001
Nutrient (Medium)	2	93.6	56.7	< 0.0001
Nutrient (High)	2	91.3	56.9	< 0.0001
Flood Duration	2	76.5	41.8	< 0.0001
Flood Duration (NP)	2	99.3	53.9	< 0.0001
Flood Duration (np)	2	99.8	54.2	< 0.0001
Flood Duration (Low)	2	95.5	46.7	< 0.0001
Flood Duration (Medium)	2	93.9	54.3	< 0.0001
Flood Duration (High)	2	94.5	56.7	< 0.0001
Atrazine	3	85.8	31.9	< 0.0001
Atrazine (Bi-Weekly)	3	124.9	38.1	< 0.0001
Atrazine (Monthly)	3	124.2	35.9	< 0.0001
Atrazine (NP)	3	124.1	39.1	< 0.0001
Atrazine (np)	3	124.8	37.1	< 0.0001

[1]Degrees of Freedom -Numerator; [2]Degrees of Freedom - Denominator

Plant Tissue Nutrient Content

A one-way ANOVA revealed that the carbon content in the aboveground (Stem) and belowground tissue (Roots) of *S. patens* nutrient treatments was significantly different from the

Control (Table 6.4, $F = 14.5$, $p < 0.0001$). The carbon content for the NP and np nutrient treatments in both the roots and stem was higher than Control .

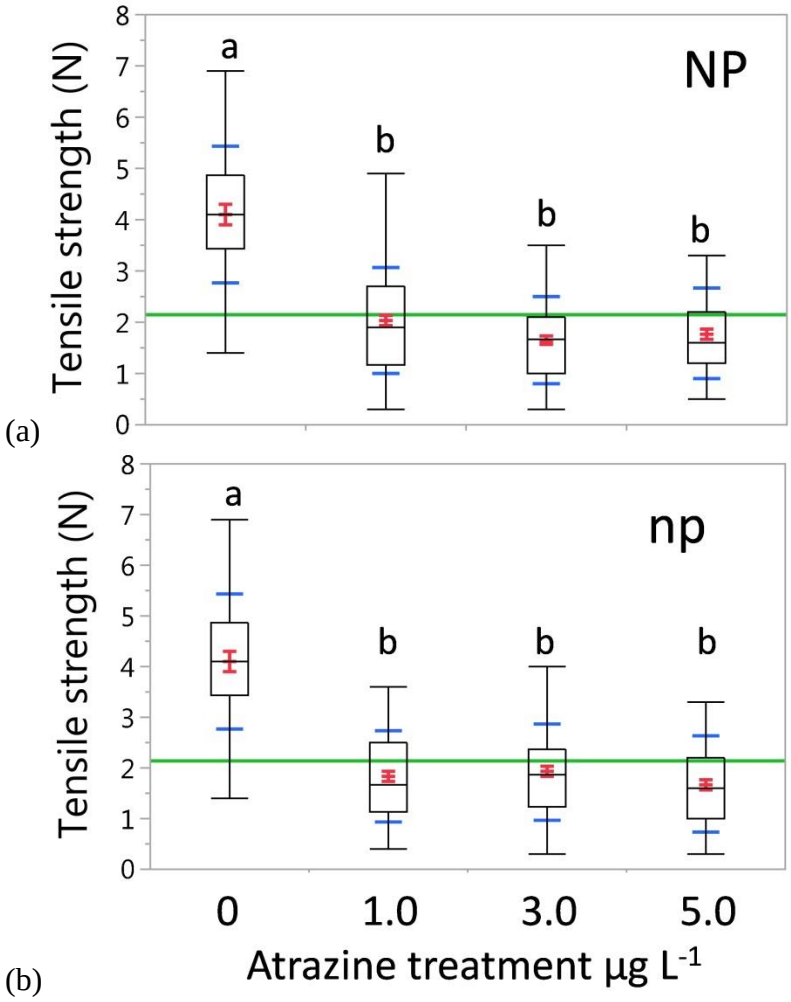


Fig. 6.8 Box and whisker plot of one-way Welch's ANOVA of tensile root strength with atrazine as the main effect for the (a) High Nitrogen-High Phosphorus (NP), and (b) Low Nitrogen-Low Phosphorus (np) nutrient addition data subsets to test for interactive effects between nutrient and atrazine treatments. There were significant differences between control and nutrient treatments (Table 6.1, 6.2; $p < 0.0001$) in both subsets. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

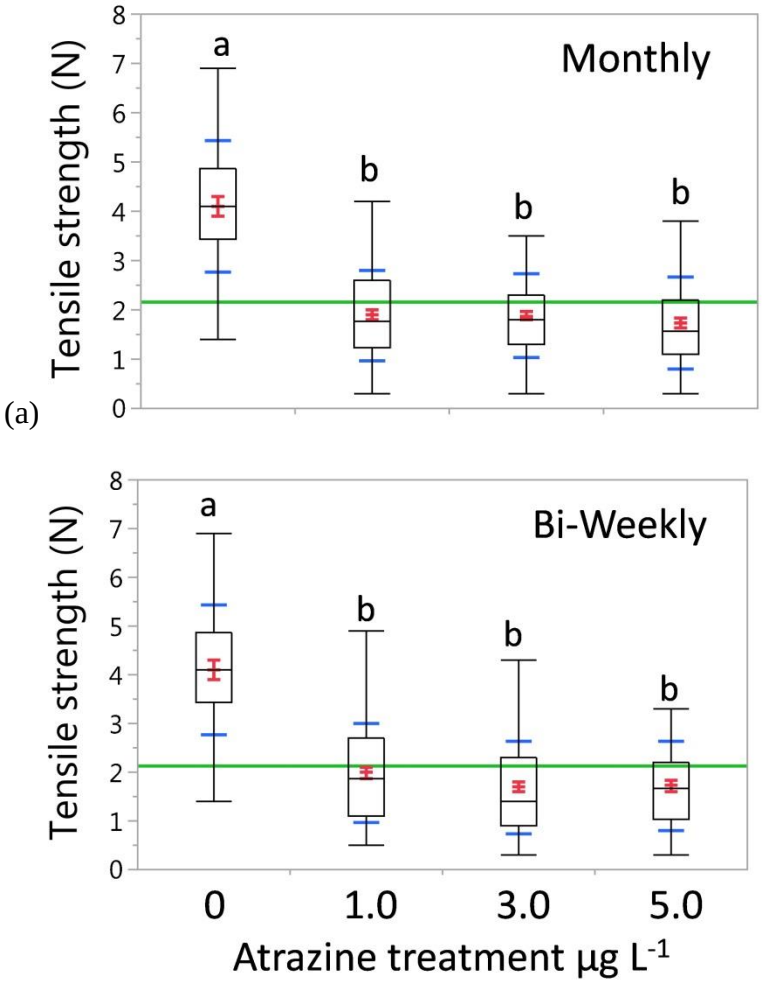


Fig. 6.9 Box and whisker plot of one-way Welch's ANOVA of tensile root strength with atrazine as the main effect for the (a) Monthly, and (b) Bi-Weekly flood duration data subsets to test for interactive effects between flood duration and atrazine treatments. There were significant differences between control and nutrient treatments (Table 6.1, 6.2; $p < 0.0001$) in both subsets. The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error; the horizontal green line across the plot is the grand mean. Box plots with different letters denote significant differences between treatments

Also, more carbon was detected in the roots than in the aboveground tissue. In the aboveground tissue, a Student's t-test found that the carbon content in the NP treatments was significantly different from the np treatments ($p < 0.0001$).

Table 6.3 Summary of mean soil parameters of a nutrient-atrazine-flood duration interaction experiment delineated by flood duration treatment. Mean values with different letter superscripts are significantly different ($p < 0.05$)

Parameter	Experimental Treatments		
	Bi-Weekly	**Monthly**	**Control**
Soil Temperature (°C)			
Mean	26.0[a]	26.2[a]	25.9[a]
Min	25.1	25.2	25.2
Max	27.0	27.0	27.0
Standard Error	0.19	0.20	0.20
pH			
Mean	7.4[a]	7.4[a]	7.0[a]
Min	7.2	7.3	6.9
Max	7.5	7.5	7.1
Standard Error	0.03	0.02	0.03
Redox Potential (mV)			
Mean	88.0[a]	86.1[a]	91.3[a]
Min	6.2	2.7	33.1
Max	166.1	164.5	159.8
Standard Error	20.8	19.9	14.5

Also, there were greater concentrations of nitrogen and phosphorus in the roots than in the stems. The nitrogen content of the nutrient treatment in both the roots and stem was higher than the Control; however, there were significant differences in nitrogen content between the aboveground and belowground tissue and Control ($p > 0.05$). The phosphorus content of the nutrient treatments in both the roots and stem was also higher than the Control; however there was no significant difference in phosphorus content between the aboveground and belowground tissue and Control ($p > 0.05$). A Student's *t*-test found that there was a significant difference in the phosphorus content between the nutrient treatments and controls in roots and the nutrient treatments and controls in the stem ($p > 0.05$).

The C:N ratio for both the roots and the stems were less than 100 (Table 6.4). In the roots, the C:N ratio ranged from 66.1 in the HN treatments to 83.4 in the Control; whereas the C:N ratio in the stem ranged from 72 in the HN treatments to 84 in the Control. In addition, the C:N ratio of the aboveground nutrient treatments was higher than those of belowground nutrient treatments. However, in the N:P ratios, the stem ratios in the nutrient treatments were higher than the root ratios. In the roots, the N:P ratios ranged from 9.8 in the NP treatment to 12.3 in the Control treatments. In the stems, the N:P ratios ranged from 17.8 in the np treatments to 28.1 in the Control

Atrazine Levels

Neither atrazine nor any of its primary metabolites were detected in leaf, root, or solid soil samples from any of the Low, Medium, or High atrazine experimental treatments (25 μg L^{-1} detection limit). The detection limit for leaf and root samples was 25 μg L^{-1}; however, the detection limit for water and porewater samples was 0.1 μg L^{-1}. Atrazine was detected in the soil porewater of the Low, Medium, and High atrazine treatments at a concentration of 0.0083 μg L^{-1}, 0.0095 μg L^{-1}, and 0.0435 μg L^{-1}, respectively. In addition, atrazine and DEA were detected in the deionized water controls at mean concentrations of 6.96 and 1.60 μg L^{-1}, respectively.

DISCUSSION

One-way ANOVAs of the three main effects indicated that the tensile root strength of *S. patens* was significantly reduced by flood duration (1.96 N), atrazine treatment (1.92 N), and nutrient addition (1.71 N) compared to the Control (4.10 N). In addition, the tensile root strength losses of the combination treatments were greater than 49% for all combinations. However, there were no significant differences in tensile root strength among the three main effects.

Table 6.4 Results of nutrient tissue content testing of live *S. patens* above- (stem) and belowground biomass (roots) for carbon, nitrogen, and phosphorus as well as carbon-nitrogen (C:N) and nitrogen-phosphorus (N:P) ratios. Mean values with different letter superscripts are significantly different ($p < 0.05$). Comparisons of means were made within each nutrient between treatments and control as well as between roots and stems

Treatment	**Carbon (mmol/g)**		**Nitrogen (mmol/g)**		**Phosphorus (mmol/g)**		**C:N**		**N:P**	
	Roots	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*	*Roots*	*Stem*
NP	37390^{c}	36883^{d}	648.5^{c}	752.3^{d}	66.0^{c}	35.1^{a}	57.7	49.0	9.8	21.4
np	37502^{c}	36808^{d}	598.4^{c}	587.7c	57.3^{c}	33.1^{a}	62.7	62.6	10.4	17.8
Control	36356^{a}	36534^{b}	502.5^{a}	399.2^{b}	41.0^{a}	14.2^{b}	72.4	91.5	12.3	28.1

In addition, there were no interactive effects detected among the three main effect. Due to the short duration of the experiment (120 days), the flood duration treatment could be expected to affect tensile root strength because of the plant's physiological adaptations to flooding. Hypoxic or anoxic conditions form in the rhizosphere as water displaces oxygen in the soil. Ethylene is a plant hormone and its formation can create a carbon demand on root tissue (Willey 2016). The formation of aerenchyma appears to immediately reduce tensile root strength because of the loss of tissue and increased root porosity, despite the recalcitrant nature of aerenchyma. As the internal structure of the root is altered, its ability to withstand external loading also may be altered. Before flood adaptations are deployed, the structure of the root resembles a semi-solid column with numerous horizontal and vertical internal support structures that may have a greater ability to attenuate external tensional loads (Niklas and Spatz 2012).Niklas (1992) stated that it is important to think of the biomechanical properties of a plant as structures such as beams and columns, rather than tissue because forces are exerted on plant tissue from multiple vectors in three dimensions. However, the lysigenous process of lacunae formation reduces this internal support structure to create large pore spaces for gas exchange. As a result, a smaller amount of tissue assumes the load bearing capacity for the root. Stress is defined as force per unit area and root volume can increase with increasing porosity; therefore, with less tissue and more volume, the 'beams' and 'columns' in the root cortex may then be subjected to more force, stress, and even shear stress. The spans of the 'beams' and 'columns' increases as less structural material support the increased amount of volume. Striker et al. (2007) examined the trade-off between aerenchyma formation and root mechanical strength in four emergent macrophytes. They concluded that unless the remaining tissue had been reinforced by sclerenchyma, the tensile root strength decreased considerably with increasing porosity regardless of the species. It is unknown

how the formation of aerenchyma affects the alignment of macro- and microfibrils, which are the primary, cellulose-rich support elements within the tissue. The alignment of the macrofibrils can affect tensile root strength and even a change in turgor pressure can influence the alignment of the macrofibrils. Cronk and Fennessy (2001) stated that aerenchyma formation may reduce the internal root tissue (parenchyma) by 60% or more. Also, aerenchyma formation may continue as the redox potential decreases, which would further increase root porosity. The redox potential for the flood duration treatments dropped to a minimum of +6.7 ± 20.8 mV for the Bi-Weekly treatment and +2.7 ± 19.9 mV for the Monthly treatment. Consequently, as the soil oxygen levels dropped below the aerobic-anaerobic threshold of +300 mV, aerenchyma tissue formation and root porosity could have increased and facilitated a corresponding reduction in tensile root strength.

The increase in root porosity due to flood adaptations may have been exacerbated by the effects of nutrient addition. The addition of calcium nitrate tetrahydrate [$Ca(NO_3)_2 \bullet 4H_2O$] provided nitrate as an electron acceptor to facilitate metabolic functions and drive nutrient cycling processes such as denitrification. Organic carbon was the electron donor, which may have reduced root biomass. Radial oxygen loss from the roots may not occur along the entire surface area of the root; consequently, nitrate may be used as an alternate electron acceptor during respiration. In addition, the diffusion of oxygen from root to shoot encounters respirative tissue from the root tip to the atmosphere, which could have resulted in addition biomass loss due to aerobic respiration inside the root. The soil temperature increased by 2 °C from June to July, which may have increased metabolic reaction rates. However, nitrogen and phosphorus were added together to the experimental treatments. The phosphorus content in the roots was nearly twice the content in the stem. Also, the N:P ratios for both nutrient treatments were below

15, which is an indication of nitrogen limitation. Nitrogen may have been the 'limiting' nutrient because nitrate may have been used as an electron acceptor – this was not known for sure. But if it was, then, a 'surplus' of phosphorus may have accumulated within the root. Phosphorus is a macronutrient that is important for root growth. The resultant effect of available phosphorus may have been the inhibition of addition root growth. As the redox potential dropped to near zero and as root porosity increased, then additional biomass may not have been generated due to the presence of phosphorus and the concomitant loss of carbon as an electron donor. Root foraging may be curtailed with nutrient levels in excess of the plant's need (McNickle and Cahill 2009). However, in a 25-month greenhouse experiment, Poormahdi (2014) added NH_4Cl (8.3 g N m^{-2} yr^{-1}), nitrate in form of $Ca(NO^3)_2 \bullet 4H_20$ (8.3 g N m^{-2} yr^{-1}), phosphate in form of KH_2PO_4 (0.42 g P m^{-2} yr^{-1}), sulfate in form of $MgSO_4 \bullet 7H_2O$ (75 g S m^{-2} yr^{-1}), and combination of all four nutrients to 30 cm diameter marsh sods dominated by *Sagittaria lancifolia* on a monthly basis. She found no significant effects of nutrient addition on the belowground biomass standing crop or on soil shear strength; but she did note that soil strength declined with depth, even though the differences between depths (5, 10, 15 cm) were not statistically significant. Conversely, Darby and Turner (2008b) reported that the belowground biomass of *Spartina alterniflora* live roots was reduced in 12 out 13 fertilized sites in Massachusetts, Virginia, and Louisiana. They added that there was a 49% decline in the live belowground biomass at sites with the highest belowground live biomass. Turner (2011) found a decrease in soil shear strength below the depth of 50 cm in salt marsh plots with N+P nutrient addition. The disparity in results between this study and Poormahdi (2014) in regard to effects on belowground biomass may be explained by the size of the experimental treatments and response variables. The response variable for this greenhouse study was tensile root strength. Poormahdi (2014) used sods that were 30 cm in

diameter and 25 cm in depth (750 cm^3 of belowground biomass) from fully developed marsh plants; whereas, this greenhouse study utilized seedlings with only 10.2 cm^3 of belowground biomass. Also, the scale of this greenhouse study examined the effects of multiple stressors on individual roots. Degradation of the belowground biomass may not always manifest as a reduction in mass. Furthermore, this greenhouse study demonstrated that the belowground biomass may remain essentially intact when exposed to nutrient addition but be catastrophically weak when it is evaluated in the context biomechanical forces. In an earlier experiment conducted by this author; a combined nutrient and atrazine treatment clearly demonstrated a decrease in the belowground biomass and tensile root strength of *S. patens* after a cumulative dose of 21 µg L^{-1} was administered over a 7-week period. Consequently, the negative effects of atrazine on tensile root strength may be the result of cumulative effects over time. Each dose of atrazine may further impair the plant's ability to fix carbon via photosynthesis, which may deprive the plant of the ability to maintain biomass that may be lost by metabolism and nutrient cycling. Aerenchyma tissue in some species can contain a large component of refractory material such as cellulose. In addition, the endodermis and epidermis in some species can contain sclerenchyma tissue, which may reinforce aerenchyma and provide additional tensile strength (Striker et al. 2007). Despite these measures, the adaptation to facilitate gas exchange may come at the expense of structural integrity as a carbon demand is exerted upon the roots due to respiration, processes such as denitrification, and possible tissue damage inflicted by oxygen radicals that are generated by atrazine and flooding.

CONCLUSIONS

The tensile root strength of *S. patens* was decreased by exposure to multiple stressors consisting of excess nutrient addition, atrazine, and flood duration. Adaptation to flooding

through aerenchyma formation may have directly resulted in the loss of tensile root strength, which was reduced further as carbon was as an electron donor. In addition, ethylene and ethanol production within the roots may have produced another source of carbon demand on the roots and decreased the amount of tissue in the roots, while atrazine exposure curtailed the plants' ability to fix additional carbon for maintenance. As a result, tensile root strength may decline over time as these stressors continue to reduce the amount of biomass in the root. This condition may be further modified by changes in soil temperature, pH, and redox potential - higher temperatures increase the rate of metabolic reactions; alkaline conditions can release phosphorus and increase the availability of atrazine. Also, the root porosity may increase as the plant responds to a lower redox potential by generating more aerenchyma for gas exchange. Plants exposed to atrazine for 50-days atrazine exposure experiment had changed in tensile root strength and a 60-day nutrient addition experiment did not produce significant differences in tensile root strength compared to the Control. However, if the effects of these stressors are prolonged, especially in concert with each other, the fitness of the species may be greatly reduced and the wetland ecosystem may be vulnerable to large disturbances such as tropical cyclones. In another experiment, the combination of nutrients and atrazine significantly reduced tensile root strength more than either of these treatments alone over a period of seven months. In this experiment, which lasted four months, the addition of flood duration appears to have lowered the resistance of *S. patens* enough so that the negative impacts of atrazine exposure and nutrient addition emerged. Results indicate that there may be a threshold in which the ecosystem will transition to another state of equilibrium in which the wetland-dependent organisms do not survive as the accumulated organic peat is exposed, weakened, and collapses and many ecosystem services and functions are lost. The negative effects of multiple stressors may

complicate management efforts because of the absence of interactive effects. If one of the three main effects affected one or the other two main effects, plant stress could be reduced by mitigating or eliminating the other stressor. The absence of interactive effects was an indication that the main effects were acting independently of each other. Consequently, all three stressors would have to be managed simultaneously to reduce the risk to the ecosystem. Therefore, reducing the impacts from multiple stressors would seem to be a more effective management strategy than restoration in these regards.

REFERENCES

Armstrong W, Brandle R, Jackson MB (1994) Mechanisms of flood tolerance in plants. Acta Botanica Neerlandica 43(4):307-358

Brady NC, Weil RR (2002) The Nature and Properties of Soils, 13th edition. Prentice Hall Upper Saddle River, New Jersey 960 pp.

Colmer TD, Voesenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. Functional Plant Biology 36:665–681

Cronk JK, Fennessy MS (2001) Wetland Plants: Biology and Ecology. CRC Press, New York, NY 462 pp.

Crowell M, Coulton K, Johnson C, Wescott J, Bellomo D, Edelman S, Hirsch E (2010) An estimate of the U.S. population living in 100-year coastal flood hazard areas. Journal of Coastal Research 26(2):201-211

Darby FA, Turner RE (2008a) Below- and aboveground biomass of *Spartina alterniflora*: Response to nutrient addition in a Louisiana salt marsh. Estuaries and Coasts 31(2):326-334.

Darby FA, Turner RE (2008b) Effects of eutrophication on salt marsh root and rhizome biomass accumulation. Marine Ecology Progress Series 363:63–70.

Dat J, Vandenabeele S, Vranova´ E, Van Montagu M, Inze´D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cellular and Molecular Life Sciences 57:779–795

Deegan LA, Johnson DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM (2012) Nature 490:388-392

Evans DE (2003) Aerenchyma formation. New Phytologist.161:35–49

Fiedler S, Vepraskas MJ, Richardson JL (2007) Soil redox potential: Importance, field measurements, and observations. Advances in Agronomy 94:2–56

Fufezan C, Rutherford AW, Krieger-Liszkay A (2002) Singlet oxygen production in herbicide-treated Photosystem II. FEBS Letters 532:407-410

Ghosh PK, Philip L (2006) Environmental significance of atrazine in aqueous systems and its removal by biological processes: An overview. Global NEST Journal 8(2):159-178

Graham SA, Mendelssohn IA (2014). Multiple levels of nitrogen applied to an oligohaline marsh identify a plant community response sequence to eutrophication. Marine Ecology Progress Series 417:73–82.

Graham SA, Mendelssohn IA (2016). Contrasting effects of nutrient enrichment on below-ground biomass in coastal wetlands. Journal of Ecology 104:249–260.

Hollis LO, Turner RE (2018) The tensile root strength of five emergent coastal macrophytes. Aquatic Botany (in press)

Jackson CR (2006) Wetland hydrology *In:* DP Batzer, RR Sharitz (eds.) Ecology of Estuarine and Freshwater Wetlands. University of California Press, Los Angeles, CA 568 pp.

Justin SHFW, Armstrong W (1987) The anatomical characteristics of roots and plant response to soil flooding. New Phytologist 106:465–495

Keddy PA (2010) Wetland Ecology: Principles and Conservation. Cambridge University Press, New York, NY 497 pp.

Krieger-Liszkay A, Rutherford AW (1998) Influence of herbicide binding on the redox potential of the quinone acceptor in Photosystem II: Relevance to photodamage and phytotoxicity. Biochemistry 37(50):17339-17344

Kozlowski TT (1984) Plant responses to flooding of soil. BioScience, 34(3):162-167

McNickle GG, Cahill, Jr. JF, (2009) Plant root growth and the marginal value theorem. Proceedings of the National Academy of Sciences 106(12): 4747–4751

Mitsch WJ, Gosselink JG (2000) Wetlands, Third Edition. John Wiley and Sons, Inc. New York, NY 920 pp.

Morris JT, Bradley PM (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. Limnology and Oceanography 44(3):699–702

Naidoo G, McKee KL, Mendelssohn IA (1992) Anatomical and metabolic responses to waterlogging and salinity in *Spartina alterniflora* and *S. patens* (Poaceae). American Journal of Botany 79(7):765-770

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago, IL 608 pp.

Niklas KJ, Spatz HC (2012) Plant Physics. University of Chicago Press, Chicago, IL 426 pp.

Poormahdi S (2014) Nutrient and Sediment Effects on Oligohaline Marsh Plant Community in Mississippi River Delta. Master's thesis, Louisiana State University Baton Rouge, LA 55 pp.

Reddy KR, Delaune RD (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, New York, NY 774 pp.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Hindawi Publishing Corporation Journal of Botany 2012:1-26

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Pointe TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall, Jr. LW, Williams WM (1996) Ecological risk assessment of atrazine in North American surface waters. Environmental Toxicology and Chemistry 15(1):31–76.

Striker GG, Insausti P, Grimoldi AA, Vega AS (2007) Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. Plant, Cell and Environment 30:580–589.

Striker GG (2012) Gustavo Gabriel Striker (2012). Flooding Stress on Plants: Anatomical, Morphological and Physiological Responses, *In*: J Mworia (Ed.) Botany. InTech Europe Rijeka, Croatia pp. 1-28.

Swarzenski CM, Doyle TW, Fry B, Hargis TG (2008) Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. Biogeochemistry 90:49–63

Turner RE, Howes BL, Teal JM, Milan CS, Swenson EM, Goehringer-Toner DD (2009) Salt marshes and eutrophication: An unsustainable outcome. Limnology and Oceanography 54(5):1634–1642.

Turner RE (2011) Beneath the salt marsh canopy: Loss of soil strength with increasing nutrient loads. Estuaries and Coasts 33:1084-1093. doi: 10.1007/s12237-010-9341-y.

United States Environmental Protection Agency (USEPA) (2016) Refined Ecological Risk Assessment for Atrazine. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington D.C.

Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: Limnology and Oceanography (21)2:245-252

Wigand C, Brennan P, Stolt M, Holt M, Ryba S (2009) Soil respiration rates in coastal marshes subject to increasing watershed nitrogen loads in southern New England, USA. Wetlands 29(3):952–963.

Willey N (2016) Environmental Plant Physiology. Garland Science, Taylor and Francis Group, LLC New York, NY 390 pp.

Zhu J, Patzoldt WL, Radwan O, Tranel PJ, Clough SJ (2009) Effects of Photosystem-II-interfering herbicides atrazine and bentazon on the soybean transcriptome. The Plant Genome 2(2):191–205

CHAPTER 7
ECOLOGICAL AND MANAGERIAL IMPLICATIONS OF IMPAIRED TENSILE ROOT STRENGTH OF EMERGENT COASTAL WETLAND MACROPHYTES

ECOLOGICAL IMPLICATIONS

Emergent macrophytes may function as keystone species in wetland and aquatic ecosystems. They are primary producers that form the foundation of food webs by providing forage for invertebrate and vertebrate species. In addition, emergent macrophytes are a source of organic carbon for bacteria and their above- and belowground biomass can serve as a substrate for periphyton. They provide the structural stability that allows coastal wetlands to occupy a position between marine and terrestrial habitats. Emergent macrophytes can influence wetland development by altering hydrology, trapping suspended sediment, and providing habitat for ecosystem engineers such as alligators and beaver. Coastal wetlands serve as vital nursery habitat for marine and estuarine fishes and invertebrates, many of which are commercially valuable species such as brown (*Farfantepenaeus aztecus*) and white (*Litopenaeus setiferus*) shrimp, blue crab (*Callinectes sapidus*) , oysters (*Crassostrea virginica*), redfish (*Sciaenops ocellatus*) , speckled trout (*Cynoscion nebulosus*), and flounder (*Paralichthys lethostigma*). In addition, coastal wetlands provide wintering and stopover habitat for numerous species of waterfowl, Neotropical songbirds, wading birds, and shorebirds. Therefore, the erosion of coastal wetlands can have profound ecological consequences such as the disruption of trophic dynamics and biogeochemical cycling and functions. The degradation of tensile root strength in wetland emergent macrophytes places the entire ecosystem in jeopardy. The belowground biomass of emergent species such as *S. patens* and *S. alterniflora* provide structural reinforcement of wetland soils, many of which are dominated by organic material. Wetlands can serve as a source, sink, or transformer of xenobiotics. The influxes of xenobiotics such as herbicides and nutrients provide a massive supply of alternate electron acceptors to a vast reservoir of carbon that can be

used as an electron donor. The degradation of belowground biomass and/or tensile root strength increases the vulnerability of wetlands to major natural disturbances such as tropical storms and hurricanes. Without emergent vegetation, the accumulated peat in wetland soils may collapse and expose coastal wetlands to inundation by the sea. The loss of coastal wetlands would mean the loss of the important ecological functions that they perform, such as the filtering, sequestration, and transformation of chemical compounds. Consequently, ecosystem processes may be disrupted further by affecting the phytoplankton, which are another major source of primary production. Eutrophication may facilitate a shift in phytoplankton communities as marine species displace or outcompete estuarine and fresh water species. Excess nutrients can alter phytoplankton community structure by changing algal competition for nutrients and by decreasing the availability of silica (Howarth et al. 2000). Silica may increase diatom production in the headwaters of estuaries, which results in sequestration of silica in bottom sediments as the diatoms die (Howarth et al. 2000). The increase in nitrogen and phosphorus in coastal waters, accompanied by a decline in silica, can shift N:S and P:S ratios and alter the composition of phytoplankton communities (Howarth et al. 2000, Turner et al. 2006). The disintegration of coastal wetlands would remove a significant means of improving the quality of water that flows to coastal areas, which may create a positive feedback loop that could increase the frequency and distribution of harmful algal blooms. Furthermore, eutrophic nutrient levels in freshwater inflows have been shown to increase the size and persistence of hypoxic or anoxic ‘dead zones’ in estuarine and nearshore areas. These low oxygen areas can kill or displace benthic, demersal, and pelagic species and alter trophic dynamics. Eutrophication can also increase water turbidity by stimulating the growth of epiphytes and macroalgae, which would inhibit light penetration into the water column (Zieman and Zieman 1989). As a result, the lower light levels may degrade

seagrass beds and exacerbate ecological damage in estuarine and nearshore areas. The loss or degradation of seagrass beds would reduce the amount of reproduction, nursery, and foraging habitat that seagrasses provide for benthic, demersal, and pelagic communities, which could adversely affect the economic status of human coastal communities.

The loss of coastal wetlands could result in the complete collapse of stocks of commercially valuable marine and estuarine species because of the synergistic effects of eutrophication due to the loss of estuarine and nearshore nursery habitat and toxic nearshore habitat for adults. Therefore, the biophysical status of coastal emergent macrophytes is of the utmost importance for coastal and marine ecosystems as well as human communities that are reliant on these resources for economic activities and personal well-being. The biomass of wetland plants are a natural defense against large natural disturbances such as tropical cyclones. However, wetlands comprised of vegetation with weak tensile root strength may be fragmented by storm surge. The loss of the important ecological service of wave attenuation could magnify the storm damage to coastal human communities, as in the case with New Orleans and the Breton Sound estuary during Hurricane Katrina. Consequently, this study has indicated that urgent action is needed to mitigate the influx of xenobiotics to coastal wetlands and estuaries.

MANAGEMENT IMPLICATIONS

Coastal areas may be the de facto receiving basins for xenobiotics and toxicants from inland areas upstream. For example, the Mississippi River watershed drains 41 percent of the contiguous United States, which includes the flow from several major river systems, including the Missouri/Platte River Basin, the Ohio/Tennessee River Basin, and the Arkansas/Red/White River Basin. Nutrients, pesticides, herbicides, heavy metals, petroleum byproducts, personal care products, sediment, and other substances create poor water quality in the watershed that increase

biological oxygen demand and should be the primary focus of coastal zone, watershed, and wetland managers. Wetlands, especially constructed wetlands, may capture, degrade, and/or transform many of these compounds. Historic wetland losses in the Mississippi River Valley exceed 50% in many of the states with land that is within the watershed of the river.

The establishment of contiguous tracts of constructed wetlands may attenuate nutrient and herbicide loads and trap or transform toxic compounds that may degrade natural wetlands. Constructed wetland cells with emergent macrophytes that are tolerant to herbicides and nutrients and a controlled hydrologic regime can, detain atrazine until the compound has completely degraded. For instance, the George W. Shannon Wetlands Project in northeast Texas diverts low quality water in the Trinity River from the Dallas-Fort Worth metroplex into an off-channel constructed wetland complex (Machingambi and Mjelde 2012). The diverted water moves through four different constructed wetland cells where toxicants may be degraded, sequestered, or transformed. After treatment, the water can be returned to the main channel of the river or pumped into a nearby reservoir that is used as a source of drinking water. Phytoremediation projects that are strategically located in upland areas can intercept and reduce nutrient and atrazine loads before they enter aquatic and wetland environments.

Phytoremediation is method that uses plants to degrade, sequester, or neutralize organic or inorganic contaminants in soil and water (Albright et al. 2013). Native prairie grasses such as *Panicum virgatum* (Switchgrass) are often used for phytoremediation because of their extensive fibrous root systems that penetrate deeply into the soil and cover a large surface area (Aprill and Sims 1990). Belden and Coats (2004) reported that *P. virgatum* and three other native grasses removed 43% of the atrazine in an experimental leachate. Phytoremediation strips that are located at terrestrial-aquatic and terrestrial-wetland interfaces may reduce the toxicity of

compounds before they enter surface conveyances and are transported farther downstream or into wetlands. In addition, the restoration of natural freshwater wetlands, both forested and herbaceous can provide additional non-point source pollution abatement.

The synchronization of various management objectives may serve to mitigate the effects of much greater challenges such as the coastal erosion of Louisiana wetlands and the presence of the large hypoxic or 'Dead Zone' in the Gulf of Mexico. The Coastal Wetlands Planning, Protection and Restoration Act (CWPPRA) and the Mississippi River/Gulf of Mexico Nutrient (MRGOMN) task forces are two federal teams that are currently engaged in solving these problems, which are directly related to the Mississippi River watershed. States within the Mississippi River Watershed should ensure that their natural resources management plans are consistent with goals of CWPPRA and the MRGOMN task force. State and local efforts to combat invasive species, create wildlife habitat, improve wildlife habitat, reestablish forests, or restore wetlands may be modified at the smaller scale to achieve objectives at a larger scale. For instance, longleaf pine savannas may be found in mid-gradient areas of southeastern U.S. watersheds; these savannas may detain overland runoff for a sufficient amount of time for soil saturation to support ephemeral wetlands. Longleaf pine savanna restoration generally consists of propagating the native longleaf pine (*Pinus palustris)* and herbaceous understory of native grasses. A project of this nature may be enhanced by selecting the most flood-tolerant native grasses with fibrous root architecture that could temporarily withstand anaerobic soil conditions. The aerobic-anaerobic interfaces of these ephemeral wetlands may trap, degrade, or transform xenobiotics from higher gradient locales.

Additional collaboration by other federal agencies with the existing federal task forces could be another effective method. For example, the United States Department of Agriculture

(USDA) administers the Agricultural Act or 'Farm Bill', which provides funding for the Conservation Reserve Program (CRP) and Wetlands Reserve Program (WRP). The CRP and WRP pay private landowners to enroll farmlands in an easement agreement with the Federal government. In the CRP, agricultural land may be taken out of production to allow for habitat restoration, while the WRP actively restores wetlands that have been degraded by farming or forestry practices. Strategic planning by the USDA, CWPPRA and MRGOMN task forces could result in restoration projects on contiguous or nearly contiguous tracts. In the upland areas, strips of grassland could perform phytoremediation of toxic substances such as pesticides and herbicides. Conversely, restored forested wetlands in the Mississippi River valley could detain and reduce nutrient loads from upstream. Conservation practices related to forestry may also be productive countermeasures against eutrophication. The expansion of streamside management zones from the current minimum widths to ecologically sensible proportions may also help improve water quality. In addition, the removal of unused logging roads may reduce soil erosion by overland flow. Local efforts to combat the proliferation and propagation of invasive species could also help improve the quality of waters flowing to the Gulf of Mexico. Floating and submerged aquatic plants may act as sinks for nutrients by assimilating them for growth and maintenance. As a result, the removal of invasive species such as *Eichhornia crassipes* (Water Hyacinth) and *Hydrilla verticillata* (Hydrilla) may remove sequestered phosphorus from riverine habitats. Ongoing control efforts to eradicate mammalian pests such as feral hogs and nutria may help improve water quality by reducing the sediment loads created by the excavations of these animals. The soil disturbances created by these animals can release xenobiotics that have been sequestered below the surface.

However, restoration and remediation efforts may be futile without a severe reduction of the nutrient and atrazine loads that emanate from the upper Mississippi River watershed. In 2011, the United States Environmental Protection Agency atrazine monitoring program in 30 community water systems in 10 states found atrazine to be above the limit of detection in 3249 of 3527 raw water samples (Albright et al. 2013). Goolsby et al. (1997), detected atrazine in 98% of surface water samples from 132 streams in the upper midwestern United States. Battaglin et al. (2000) also detected atrazine in 100% of 129 samples from 75 rivers and streams in the midwestern United States in 1998. During a major flood in 2011, atrazine was detected at 100% frequency by 13 water quality monitoring stations in the lower Mississippi River-Atchafalaya River subbasin (Welch et al. 2014). As a result, *management* of this ubiquitous contaminant does not appear to be a feasible option. Also, the presence of the large and persistent hypoxia zone along the Louisiana coast is a poignant reminder that nutrient pollution to coastal zone has not abated. Therefore, a reexamination of U.S. agricultural policies and practices will be required in order to eliminate the risk that high nutrient loads and atrazine pose to coastal wetlands. The United States Geological Survey has acknowledged that agricultural operations are one of the primary sources of excess nutrient loads and herbicides (Welch et al. 2014), yet the runoff from these areas has been classified as "non-point source pollution." As a result, agricultural producers do not pay the full environmental costs of the impacts of their use of fertilizers and pesticides. The management and restoration of aquatic, estuarine, marine, and wetland ecosystems cannot succeed unless the sources of the xenobiotics that are degrading these systems are severely curtailed and/or eliminated entirely. However, accomplishing that goal will require a new policy and regulatory paradigm that can transform the operations of the entities that are responsible for these sources of 'non-point source pollution' that can degrade coastal wetlands.

The modification of existing coastal zone management policy could lessen the impact of anthropogenic activities on wetland ecosystems. For instance, this study and others have demonstrated that prolonged residence time of flood water may be detrimental to wetland plants. The frequency and severity of flood pulses can be mitigated by reducing or eliminating anthropogenic interference with wetland hydroperiods. For instance, human real estate and industrial development in the coastal zone often involves the installation of flood control infrastructure such levees, drainage ditches, canals, detention ponds, and channelization of natural streams. In addition, the construction of roads and the installation of concrete and asphalt surfaces can increase the velocity of overland runoff, as well as direct the flow (along with nutrients and other xenobiotics) to other lower gradient areas. Consequently, prospective builders of coastal construction projects should be required to assume the full environmental costs of their activities, including any future management and/or restoration efforts that may be needed to mitigate the impact of development on coastal ecosystems.

However, there are management options in Mississippi River basin Louisiana that are unique to Louisiana: the backfilling of dredged canals, removal of spoil banks, and the attenuation of nutrient and herbicide loads in surface runoff from sugarcane fields. Dredged canals have altered the hydrology of Louisiana coastal wetlands and facilitated saltwater intrusion, which has resulted in wetland damage and losses. In addition, the concomitant spoil banks have altered natural hydrology and promoted wetland losses by trapping floodwaters and storm surges and inducing prolonged inundation of wetland plants. Removal of these anthropogenic disturbances and restoring wetlands in their place may slow the rate of erosion and coastal land loss. Sugarcane fields are a less obvious threat to coastal wetland stability. High nutrient and atrazine loads emanate from these fields during precipitation and storm events and

flow directly into the estuaries, especially into the Barataria and Terrebonne basins. The runoff from sugarcane fields occurs via surface conveyances of ditches that are only a short distance from coastal wetlands. As a result, nutrient absorption and atrazine degradation is unlikely because of the lack of contact with the soil. In addition, atrazine is unlikely to undergo photodegradation due to the turbidity of the surface runoff. Although atrazine may be degraded in the water column, there is a paucity of documentation about its fate in the soil porewater of wetlands. Furthermore, atrazine produces several metabolites, which may be as toxic to plants as the parent compound. The fluxes of atrazine are infrequent; but, the concentrations of atrazine may be several times higher than the treatments used in this study, which clearly reduced the tensile root strength of *S. patens*, the most ubiquitous emergent macrophyte in Louisiana wetlands.

REFERENCES

Albright III VC, Murphy IJ, Anderson JA, Coats JR (2013) Fate of atrazine in switchgrass–soil column system. Chemosphere 90:1847–1853

Aprill W, Sims RC (1990) Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere 20:253–265

Battaglin WA, Furlong ET, Burkhardt MR, Peter CJ (2000) Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the midwestern United States, 1998. Science of the Total Environment 248:123-133

Belden JB, Coats JR (2004) Effect of grasses on herbicide fate in the soil column: Infiltration of runoff, movement and degradation. Environmental Toxicology and Chemistry 23(9):2251–2258

Goolsby D, Thurman EM, Pomes M, Meyer M, Battaglin W (1997) Herbicides and their metabolites in rainfall: Origin, transport, and deposition patterns across the midwestern and northeastern United States, 1990-1991. Environmental Science and Technology 31:1325-1333

Howarth R, Anderson D, Cloern J, Elfring C, Hopkinson C, Lapointe B, and others (2000) Nutrient pollution of coastal rivers, bays, and seas. Issues in Ecology 7:1–16

Machingambi M, Mjelde JW (2012) Wetlands ecosystem services value in constructed wetlands: The case of the George W. Shannon Wetlands Water Reuse Project. British Journal of Economics, Management, & Trade 2(2):109–124

Turner RE, Rabalais NN, Justić D (2006) Predicting summer hypoxia in the northern Gulf of Mexico: Riverine N, P, and Si loading. Marine Pollution Bulletin 52:139–148

Welch HL, Coupe RH, Aulenbach BT (2014) Concentrations and transport of suspended sediment, nutrients, and pesticides in the lower Mississippi-Atchafalaya River subbasin during the 2011 Mississippi River flood, April through July: U.S. Geological Survey Scientific Investigations Report 2014–5100, 44 p., http://dx.doi.org/10.3133/sir20145100

Zieman JC, Zieman RT (1989) The ecology of the seagrass meadows of the west coast of Florida: A community profile. U.S. Fish and Wildlife Service Biological Report 85(7.25).155 pp.

CHAPTER 8
SUMMARY

This study examined the effects of natural and anthropogenic stressors on the biomechanical properties of emergent coastal macrophytes under field and greenhouse conditions. The objectives of this study were to 1) ascertain the status of the tensile root strength of coastal macrophytes at impaired and reference wetland sites 2) test the hypothesis that excess nutrients and the herbicide atrazine degraded the tensile strength of the dominant wetland species *S. patens* under greenhouse conditions; 3) subject *S. patens* to multiple natural and anthropogenic stressors under greenhouse conditions to determine the effects on the tensile root strength of the species, and 4) ascertain if there were any interactive effects of the various stressors on the tensile root strength of *S.patens*.

Chapter 1 is an introduction to the importance of coastal wetlands and some of the natural and anthropogenic stressors that compromise the health and biomechanical integrity of the belowground biomass. Tensile strength is the resistance of a material to tensional loads. Tensile strength may be quantitatively related to soil shear strength to ascertain the status of coastal wetland soils. Coastal wetlands are subjected to high nutrient and herbicide loads during flood events and after spring agricultural operations in the Midwest and spring and fall sugarcane planting in the Mississippi River Delta. Numerous studies have indicated that these nutrient inputs can degrade the belowground biomass of coastal macrophytes and other studies have suggested that the herbicide atrazine, which disrupts photosynthesis, may be capable of doing the same.

Chapter 2 contains results from field sampling of plants. The analysis of plants from a freshwater marsh in the Lake Pontchartrain basin indicated that the partially-treated sewage effluent that had been discharged in the wetland had decreased the tensile root strength of the

emergent macrophytes *Panicum hemitomon* and *Sagittaria lancifolia*. These plants had such weak tensile root strength that they could be easily pulled out of the soil by hand. The belowground biomass of these two species contained no dead roots and the live roots were concentrated at less than 10 cm in depth, which prevented additional testing of roots in deeper soil core sections. Additional field sampling and subsequent tensile strength testing of emergent macrophytes from the Breton Sound, and Barataria basins indicated that the belowground biomasses of dominant wetland species were impaired as well. In general, dead roots were stronger than live roots at all field sites. The mean tensile strength of *S. patens* dead root samples from the Breton Sound Yscloskey site decreased with depth from 3.7 ± 0.39 N in the 0–10 cm core, 2.1 ± 0.31 N in the 10–20 cm core, to 0.9 ± 0.48 N in the 20–30 cm core. At the 10–20 cm and 20–30 cm depth, the live and dead *S. patens* roots at the Delacroix site were stronger than those at the Yscloskey site in the Breton Sound Basin. The dead root samples of *Schoenoplectus americanus* at the Delacroix site decreased in tensile strength with depth from 3.2 ± 0.41 N in the 0–10 cm core to 1.7 ± 0.41 N in the 10–20 cm core. In addition, in the 10–20 cm core, the dead *S. americanus* roots at the Delacroix site were stronger than the live roots. The tensile root strength of *Spartina alterniflora* samples were investigated at the Port Sulphur site in the Barataria Basin and at Bayou Sauvage NWR in the Lake Pontchartrain Basin. The mean tensile strength of dead roots at Bayou Sauvage NWR decreased with depth from 2.8 ± 0.66 N in the 0–10 cm core to 1.2 ± 0.70 N in the 10–20 cm core. In the 10–20 cm core, the dead *S. alterniflora* roots at Port Sulphur were stronger than those at Bayou Sauvage NWR. Overall, the differences in tensile strength between dead and live roots may be attributed to the composition of the root tissue, decomposition status, age, and site specific factors such as temperature, pH, redox, flood

duration, nutrients, xenobiotics, and microbial communities, which can influence tensile root strength of individual species in numerous ways.

Chapter 3 summarizes the results of two greenhouse experiments in which *S. patens* was exposed to atrazine at various concentrations. In the first experiment, *S. patens* was subjected to Low (0.5 µg L^{-1}), Medium (1.5 µg L^{-1}), and High (3.0 µg $L^{-}1$) levels of atrazine treatments that were administered on a weekly basis. There were no significant differences in tensile root strength between atrazine treatments and control after 50 days of atrazine exposure. The second experiment subjected *S. patens* to different atrazine levels [Low (1.0 µg L^{-1}), Medium (3.0 µg L^{-1}), and High (5.0 µg $L^{-}1$)] with three different soil textures on monthly basis for 204 days. The results of this experiment revealed significant differences in tensile root strength between the atrazine and soil texture main effects and their respective controls. The tensile root strength in the experiment units of both main effects ranged from 30 to 50% weaker than their respective controls. There were no interactive effects on tensile root strength by the atrazine and soil texture treatments. The length of time that the plants were exposed to both treatments appeared to be the greatest influence on the results. Other key components of the experimental results were the individual soil texture affinities for atrazine, adsorption and desorption dynamics, and uptake kinetics of *S.patens*. In addition, the soil parameters of temperature, pH, and redox potential were suspected of influencing the fate of atrazine.

The effects of nutrient addition and atrazine exposure on the tensile root strength of *S. patens* were explored in Chapter 4. The first experiment consisted of disturbed controls for six levels of nutrients (HN, LN, HP, LP, Np, nP) and three levels of atrazine [Low (0.5 µg L^{-1}), Medium (1.5 µg L^{-1}), and High (3.0 µg $L^{-}1$)] that were administered twice per month for two months. The nitrogen and phosphorus nutrient treatments consisted of granular calcium nitrate

tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$] and potassium phosphate [K_3PO_4] were: High Nitrogen (HN, 5.0 mg L^{-1}), Low Nitrogen (LN, 1.75 mg L^{-1}), High Phosphorus (HP, 0.30 mg L^{-1}), Low Phosphorus (LP, 0.10 mg L^{-1}), High Nitrogen x Low Phosphorus (Np), and Low Nitrogen x High Phosphorus (nP). There were no significant differences in tensile root strength between the atrazine or nutrient treatments and their respective controls after 60 days. However, in the second experiment, *S. patens* was subjected to the same atrazine and nutrient levels at the same frequency for seven months. The results of this experiment revealed significant differences in tensile root strength between the atrazine and nutrient main effects and the control. The tensile root strength in the nutrient and atrazine experimental units was 53 to 54% lower than that of the Control. A comparison of the data distributions of the main effects and combination treatments indicated that there were interactive effects on tensile root strength by the HPxH and LPxH nutrient-atrazine combination treatments. However, there were no indications of interactive effects with the remaining 16 nutrient-atrazine combination treatments, which suggest that there were no interactive effects of the six nutrient treatments and three atrazine doses. The lowest tensile root strengths were measured in the combination of the high atrazine dose and the phosphorus (HP x H; 1.32 N) and the medium atrazine dose, Low Nitrogen-High Phosphorus combination (M x nP; 1.54 N) nutrient treatments. The possible explanations for the results of the experiment included the duration of the experiment, an increase in plant respiration rates with subsequent losses of carbon, and the partial inhibition of photosynthesis by atrazine, which may have reduced the plants' ability to generate additional roots. In addition, nutrient addition may have curtailed root growth in a manner according to the Marginal Value Theorem of the Optimum Foraging Theory, which generally states (briefly) that an organism will cease foraging if its needs are met in a resource patch. Furthermore, the results of phosphorus and NP additions

are in accordance with other studies that have found that these nutrients and nutrient combinations can degrade the belowground biomass of coastal macrophytes.

The effects of a natural (flooding) and an anthropogenic (nutrient addition) stressor on the tensile root strength of *S.patens* were examined Chapter 5 . The experiment consisted of six levels of nutrients (HN, LN, HP, LP, Np, nP) with the same composition and concentrations as the previous experiment in Chapter 4, and two levels of flooding at 50% flood duration (Weekly = 3.5 days and Bi-Monthly = 7 days). The results of this experiment revealed significant differences in tensile root strength between all levels of the flood duration and nutrient main effects and the controls. The flood duration treatments had the strongest effect on tensile root strength because of the formation of aerenchyma tissue, which may have directly weakened the roots. The tensile root strength of the nutrient treatment was 47% lower than that of the Control; whereas, the flood duration treatment tensile root strength was 43% lower than that of the Control. The tensile root strength of the HN x Bi-Monthly, Np x Bi-Monthly, and nP x Bi-Monthly combination treatments reduced the tensile root strength by 54%, 54%, and 55%, respectively. The nutrient additions appears to have had additional effects on tensile root strength because the carbon demand created redox reactions in the presence of alternate electron acceptors. However, there were no interactive effects of the flood duration and nutrient addition main effects. The effects of nutrient addition may have increased the loss of tensile root strength after the initial of aerenchyma formation.

The effects of multiple stressors, both natural and anthropogenic, were investigated in Chapter 6 The experiment consisted of two levels of nitrogen-phosphorus combinations, three levels of atrazine exposure, and two levels of flood treatments with longer duration (Bi-Weekly, for: 7 days; Monthly, for: 14 days) than the previous experiment. The results from one-way

Welch's ANOVAs indicated that flood duration had the greatest effect on the reduction of tensile root strength, which appears to have occurred because of the weakening of the roots by aerenchyma formation. In addition, tensile root strength may have been diminished by the carbon demand of respirative tissue due to the presence of nitrate as an alternate electron acceptor and the possible reduction in growth due to the inhibition of photosynthesis by atrazine. The tensile root strength of the nutrient, flood duration, and atrazine treatments were 58%, 53%, and 52% lower than that of the Control, respectively. The High Atrazine x Low Nitrogen / Phosphorus treatment reduced the tensile root strength of *S. patens* by 60%. However, there were no interactive effects of the atrazine, flood duration, and nutrient addition main effects. *S. patens* possesses adaptations to deal with short-term stress induced by abiotic factors, but the impact of additional multiple anthropogenic stressors may inflict potentially catastrophic damage on the belowground biomass in a short amount of time.

Chapter 7 explored the ecological and management implications of the results of this study, which may entangle wetland scientists and policymakers in a significant quandary. The presence of toxic anthropogenic compounds within the hydrologic inputs of coastal wetlands greatly complicates wetland restoration efforts in areas such as the Mississippi River Delta. On the one hand, the river is integral to the health and survival of coastal Louisiana wetlands; on the other hand, the water in the Mississippi River possesses xenobiotics such as added nutrients and herbicides (and other compounds) that inflict chronic stress that may be harmful to coastal macrophytes over time. Wetland restoration efforts in the Mississippi River Delta and other areas will require a holistic, landscape level approach that encompasses the entire watershed and attenuates or eliminates the sources of toxicants that are degrading the water quality of the river, and, as this study has indicated, the belowground biomass of emergent coastal macrophytes.

APPENDIX A. CHAPTER 3 SOIL PARAMETER RESULTS

SOIL TEMPERATURE

An analysis of variance revealed no significant difference between the soil temperatures among the three soil textures (Figure A1a, $p > 0.05$). The highest temperature was observed in the Sand (29.1°C) units and the lowest temperature was recorded in the Organic units (23.4°C). Soil temperature in the experimental units decreased sharply in December 2015 to January 2016 and remained near 25 °C for the duration of the experiment. The soil texture experimental controls exhibit a similar pattern as the experimental units. An analysis of variance revealed no significant differences among the soil texture controls and the disturbed control (Table 3.2, $p > 0.05$). Soil temperature in the control units decreased sharply from December 2015 to January 2016 and remained near 25 °C for the duration of the experiment. The soil temperature in the control units ranged from 23.4 (± 0.19 °C) in the Control Organic units to 27.9 (± 0.19 °C) in the Control Clay units with a mean of 25.4 (± 0.19 °C) for all control units (Fig. A1b).

SOIL pH

An analysis of variance revealed significant differences between the soil pH among the three soil textures (Table 3.2; Fig. A2a; $p < 0.05$). The pH of the Organic units remained consistently below 6.0, while the Clay and Sand units fluctuated above and below pH 6.0. The mean pH of the Organic, Clay, and Sand units were 6.0 (± 0.02), 6.0 (± 0.02), and 5.9 (± 0.01), respectively. Similarly, the pH of the control units also remained acidic throughout the experiment and ranged from 4.7 in the Control Organic units to 6.2 in the Control Clay units (Fig. A2b). Also, there were significant differences in soil pH among the soil texture controls and the disturbed controls (Table 3.2, $p < 0.05$).

(a) (b)

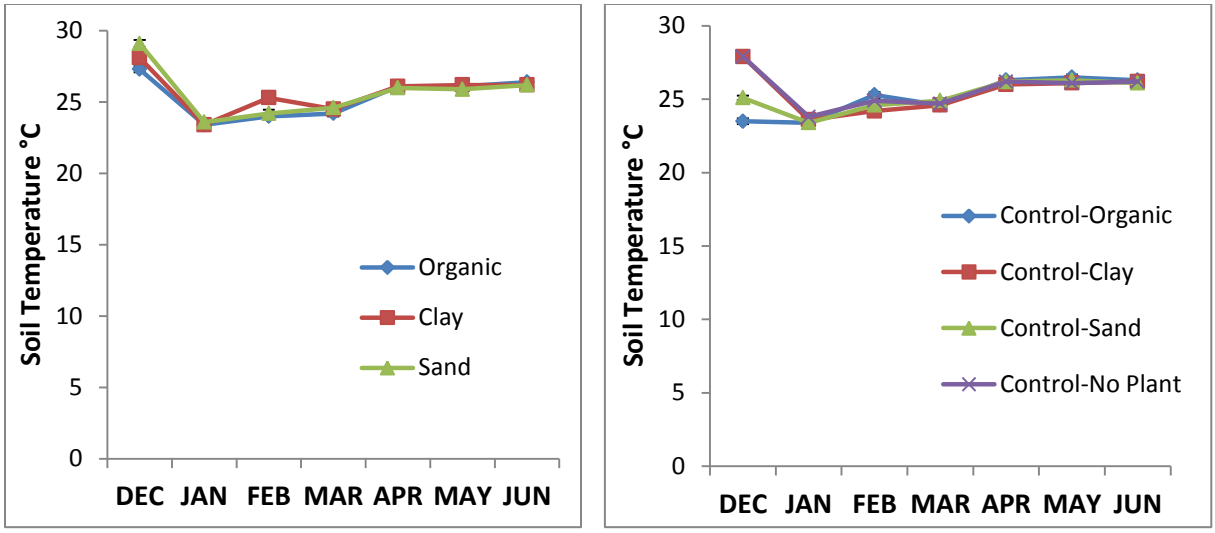


Fig. A1 (a) The monthly mean soil temperatures for the soil texture experimental units, and (b) the control units in the second 204-day atrazine experiment

(a) (b)

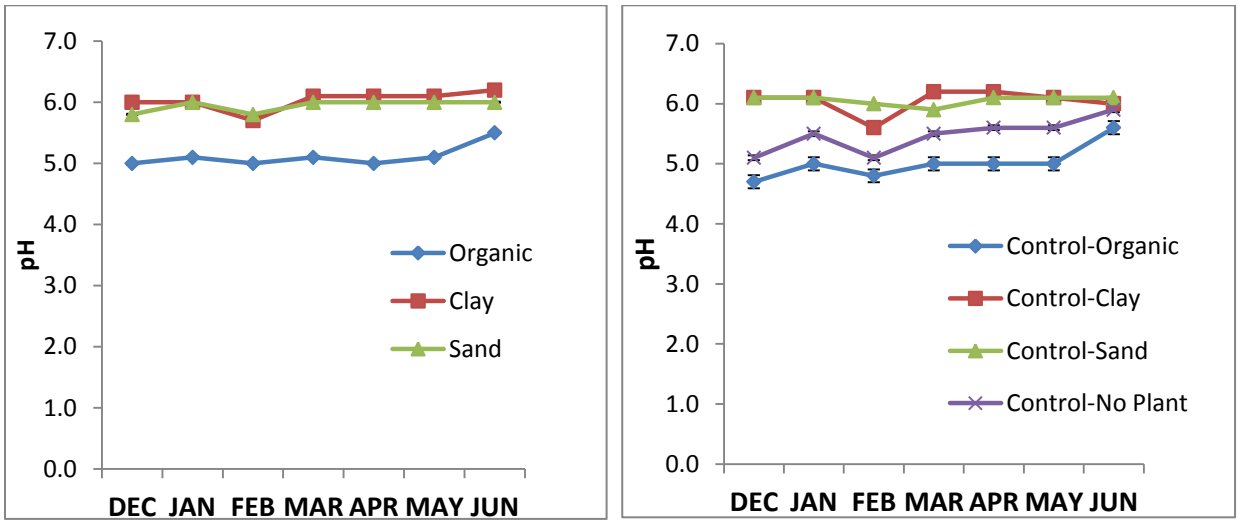
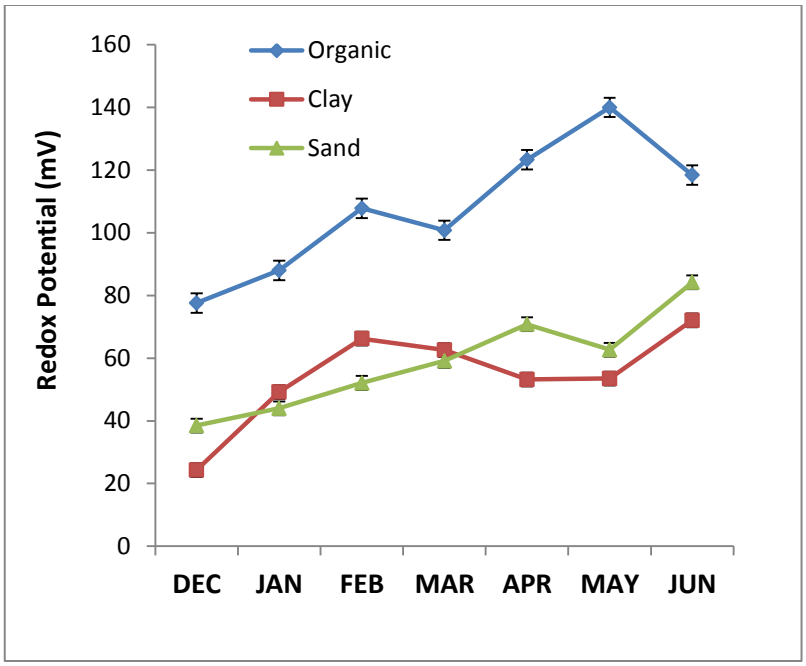


Fig. A2 (a) The monthly mean pH for the soil texture experimental units, and (b) the control units in the second 204-day atrazine experiment

(a)



(b)

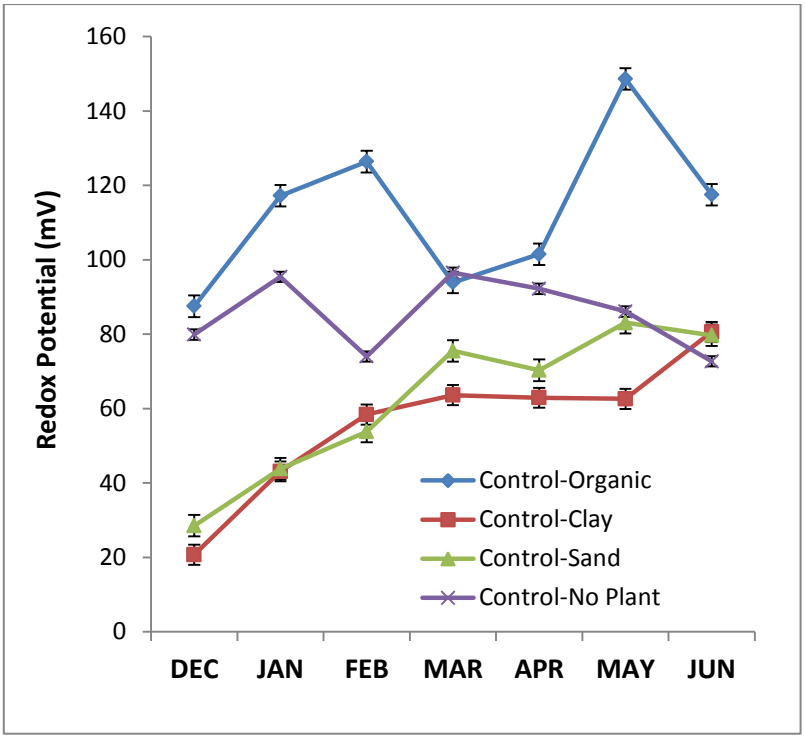


Fig. A3 (a) The monthly mean redox potential for the soil texture experimental units, and (b) the control units in the second 204-day atrazine experiment

REDOX POTENTIAL

An analysis of variance revealed significant differences between the soil redox potential among the three soil textures (Table 3.2, Fig. A3a; $p < 0.05$). The Organic experimental units ranged from +77.6 to +118.4 (± 3.1 mV) from December 2015 to June 2016, with a mean redox potential of 108.0 mV (± 3.1 mV). The Clay and Sand experimental units had similar ranges and exhibited lower redox potential than the Organic units. The Clay and Sand units ranged from +24.3 to +72.1 (± 2.2 mV) and +38.5 to +84.2 (± 2.3 mV), respectively; with mean redox potentials of +54.1 (± 2.2 mV), and +58.8 (± 2.3 mV), respectively.

The redox potentials of the control units were similar in range and magnitude to the experimental units. An analysis of variance revealed significant differences in redox potential among the soil texture controls and the disturbed control (Table 3.2, Fig. A3b; $p < 0.05$). For instance, the redox potential range of the Organic control units was +87.5 to +148.6 (± 3.0 mV), while the Clay and Sand control units ranged from +20.7 to +80.6 (± 2.7 mV) and +28.5 to +83.1 (± 2.9 mV), respectively. The mean redox potential for the Organic, Clay, and Sand units were +113.2 (± 3.0 mV), +56.0 (± 2.7 mV), and +62.1 (± 2.9 mV), respectively. However, the redox potential of the Control-No Plant units occupied a smaller range from +72.7 to +96.6 (± 1.4 mV), with a mean redox potential of 85.3 (± 1.4 mV).

APPENDIX B. CHAPTER 4 SOIL PARAMETER RESULTS

SOIL TEMPERATURE

A Student's t-test revealed no significant difference between the soil temperatures among the three atrazine treatments or Control (p >0.05). The mean soil temperature in the experimental units ranged from 26.1 to 26.6°C (Table 4.1, Fig. B1) with an overall mean of 26.3 (± 0.41 °C, SE) and less than 1°C variation between the mean temperature for each soil texture. The highest temperature was observed in the Low (31.4°C) units and the lowest temperature was recorded in the Control units (31.1°C). Soil temperature in the experimental units decreased sharply in December 2015-January 2016 and April-May 2016, but remained within 1°C of the mean temperature for most of the experiment.

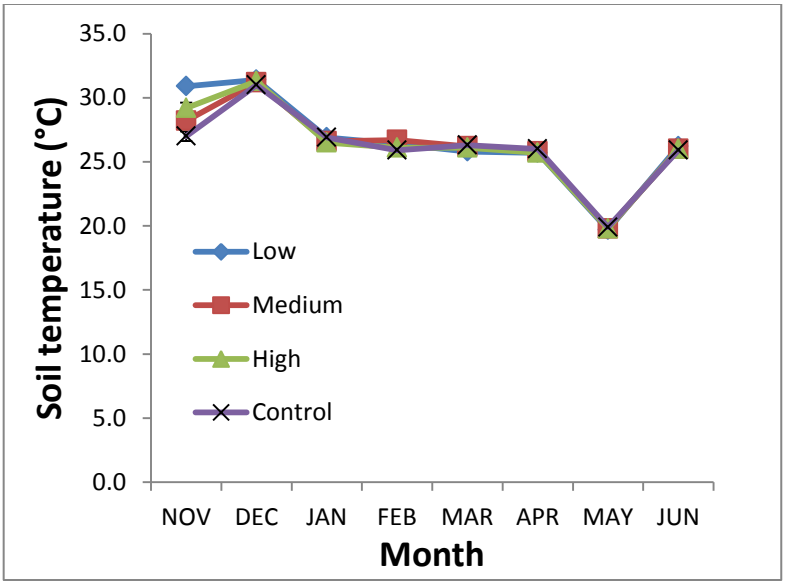


Fig. B1 The monthly mean soil temperatures for the experimental units in the 212-day atrazine-nutrient interaction experiment

SOIL pH

A Student's t-test found no significant differences between the soil pH among the three atrazine treatments and the Control (p>0.05). The pH of the experimental units was neutral to

alkaline throughout the experiment while the control units were slightly acidic for two periods in January and March 2016 (Fig B2). However, the pH of both the experimental and Control units fluctuated considerably above pH 7.0 in April-June 2016. The mean pH was 7.1 in all three atrazine treatments and the control.

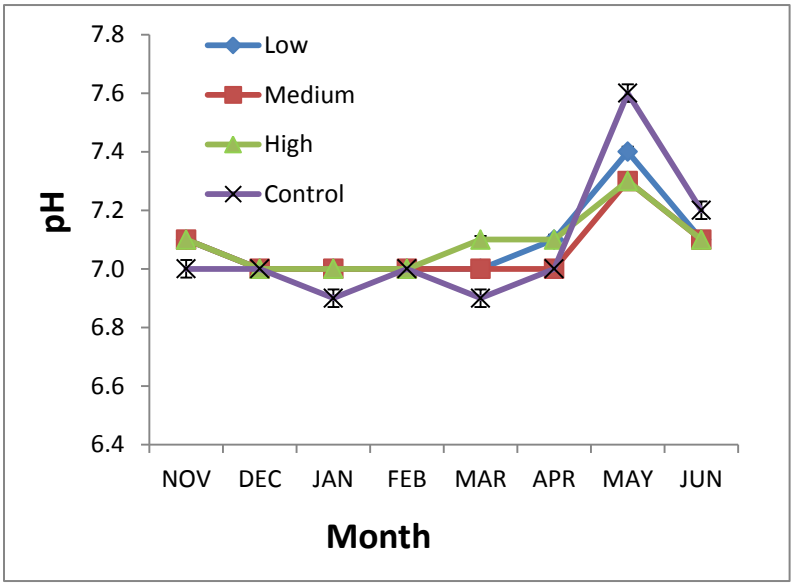


Fig. B2 The monthly mean pH for the experimental units in the 212-day atrazine-nutrient interaction experiment

REDOX POTENTIAL

The redox potential fluctuated frequently between the experimental units throughout the duration of the experiment. There was less than 6 mV of variation between the redox potential means of the experimental units and Control (Fig B3). Consequently, a Student's t-test revealed no significant differences in the soil redox potential among the three atrazine treatments and control (p >0.05).The experimental units exhibited a range differential from 15 to 25 ± 0.7 mV. For example, the Low atrazine treatment units ranged from a minimum of -26.6 mV to a maximum of -3.6 mV, which is a difference of 23 mV. The redox potentials of the experimental

and Control units remained in a range below zero throughout the experiment that was conducive to the utilization of iron and manganese as alternate electron acceptors.

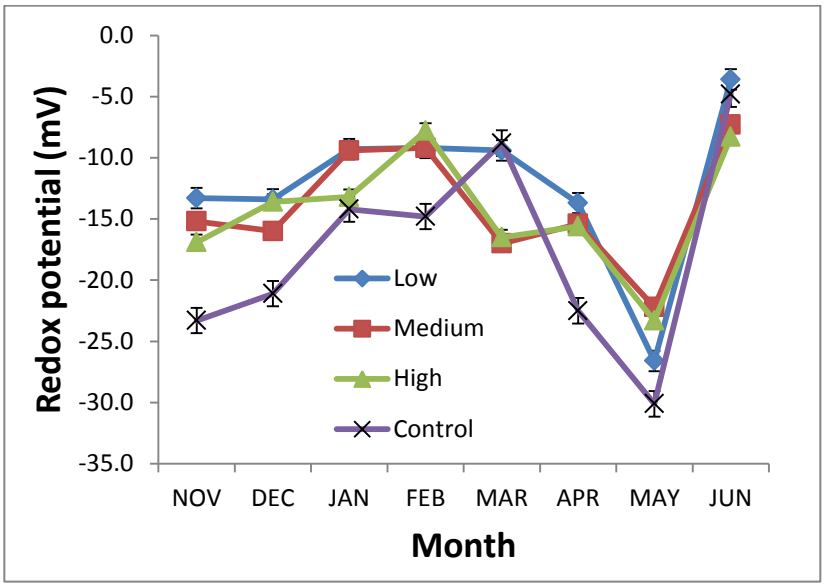


Fig. B3 The monthly mean redox potential for the experimental units in the 212-day atrazine-nutrient interaction experiment

APPENDIX C. CHAPTER 5 SOIL PARAMETER RESULTS

SOIL TEMPERATURE

The mean soil temperature in the experimental units ranged from 26.1 to 26.6°C (Table 5.1, Fig. C1) with an overall mean of 26.3 ± 0.41 °C. A Student's t-test revealed no significant difference tensile root strength among the soil temperatures or between the two flood duration treatments or control (p >0.05). The highest temperature was observed in the Bi-Monthly (31.4°C) units and the lowest temperature was recorded in the Control units (31.1°C). Soil temperature in the experimental units decreased sharply in January-February 2015, but remained within 1°C of the mean temperature for most of the experiment.

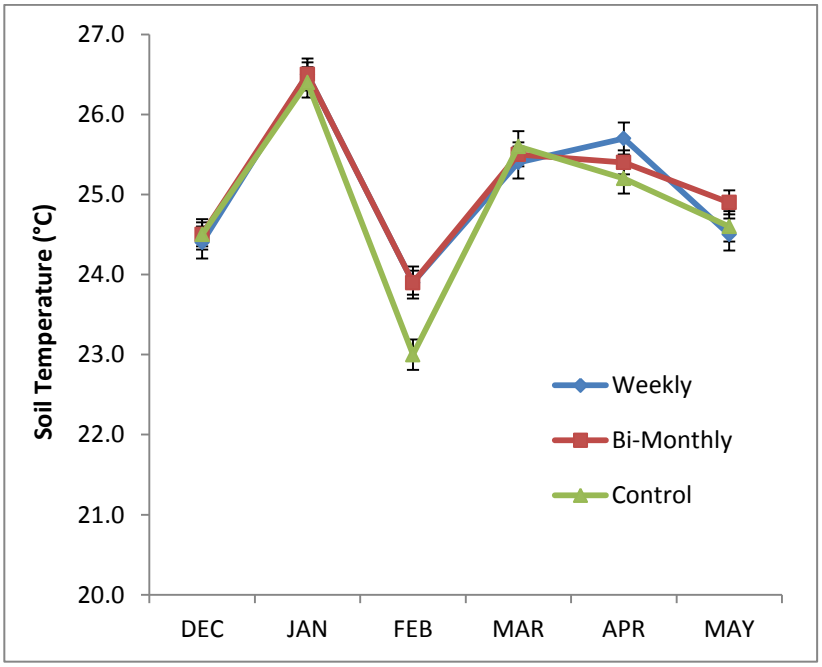


Fig. C1 The monthly mean soil temperatures for the flood duration experimental units and the control in the 165-day nutrient addition-flood duration interaction experiment. There were no significant differences in soil temperature between the treatments or control (p > 0.05)

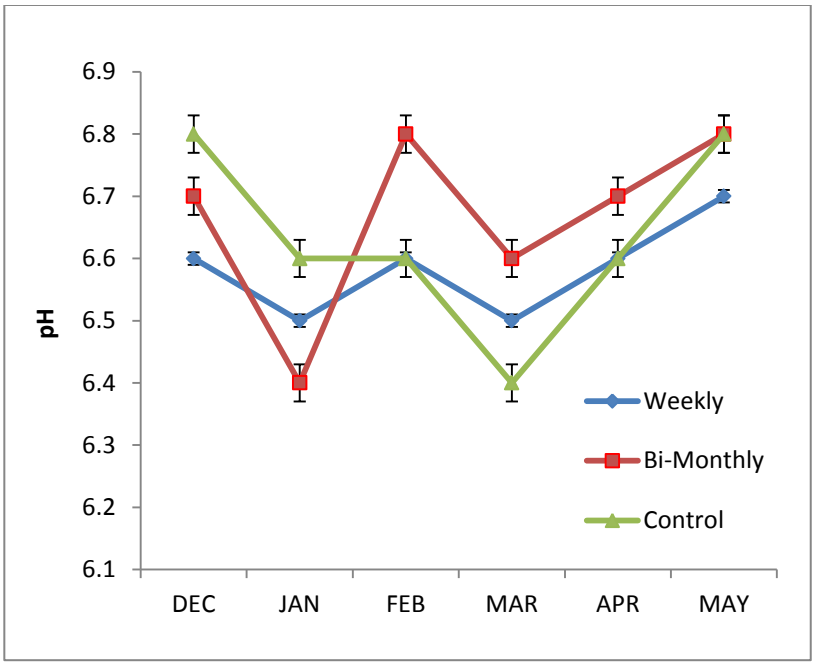


Fig. C2 The monthly mean pH for the flood duration experimental units and the control in the 165-day nutrient addition-flood duration interaction experiment. There were no significant differences in pH between the treatments or control ($p > 0.05$)

SOIL pH

The pH of the experimental units acidic throughout the experiment and the mean pH were 6.7 in both flood duration treatments and the Control (Fig. C2, Table 4.1). As a result, a Student's t-test found no significant differences between the soil pH among the in both flood duration treatments and the control ($p>0.05$). However, the pH of both the experimental and Control units fluctuated considerably dropped to pH 6.5 in March-April 2016.

REDOX POTENTIAL

The redox potential fluctuated frequently between the experimental units throughout the duration of the experiment .A Student's t-test revealed no significant differences in the soil redox potential among the flood duration treatments and Control (Fig. C2, Table 4.1; $p>0.05$). The experimental units exhibited a range differential from 33.4 to 50.9 (± 0.7 mV). For example, the Weekly flood duration treatment units ranged from a minimum of +19.2 mV to a maximum of

+55.1 mV, which is a difference of 34.8 mV. The redox potentials of the experimental and Control units remained in a range below zero throughout the experiment that was conducive to the utilization of iron and manganese as alternate electron acceptors.

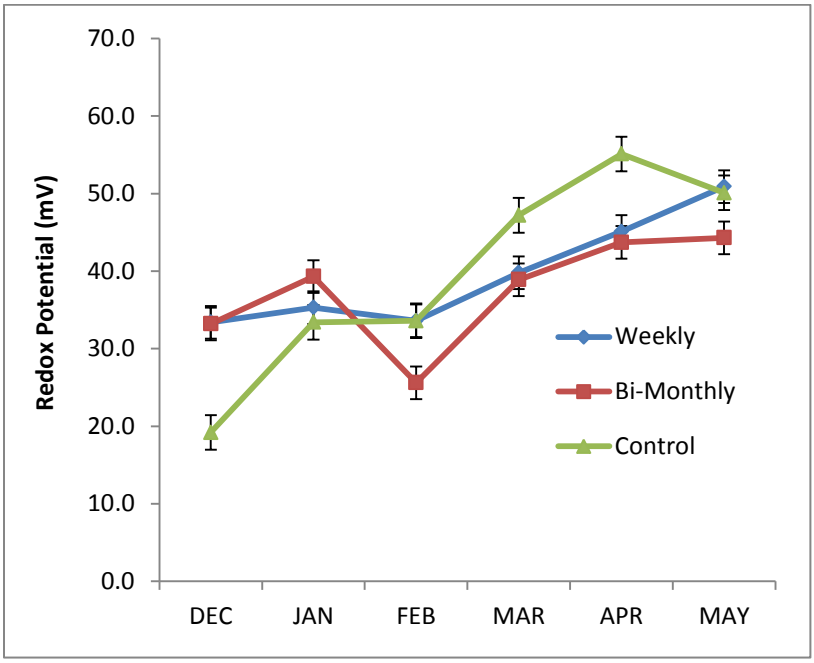


Fig. C3 The monthly mean redox potential for the flood duration experimental units and the control in the 165-day nutrient addition-flood duration interaction experiment. There were no significant differences in redox potential between the treatments or control ($p > 0.05$)

APPENDIX D. CHAPTER 6 SOIL PARAMETER RESULTS

SOIL TEMPERATURE

The mean soil temperature in the experimental units ranged from 26.1 to 26.6°C (Table 6.1, Fig. D1) with an overall mean of 26.1 ± 0.20 °C.

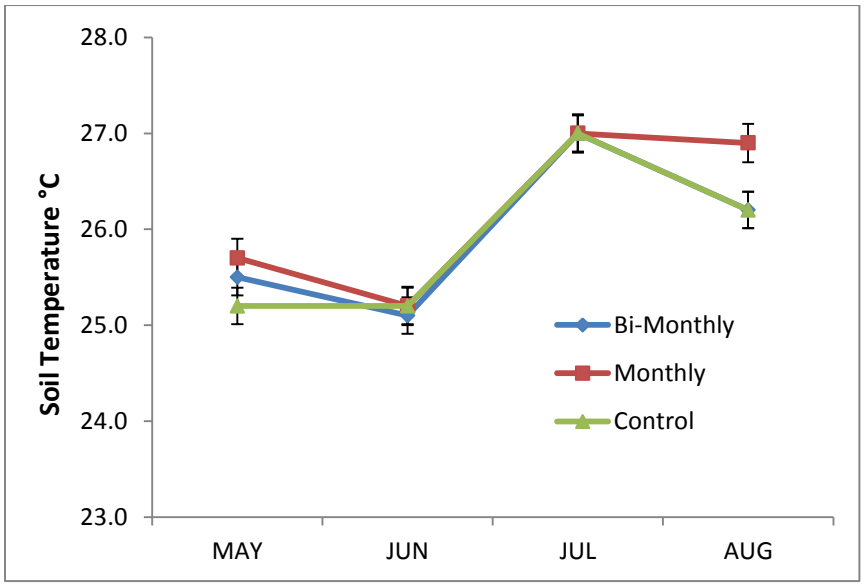


Fig. D1 The monthly mean soil temperatures for the flood duration experimental units and the control in the 123-day nutrient addition-atrazine-flood duration interaction experiment. There were no significant differences in soil temperature between the treatments or control ($p > 0.05$)

The highest temperature (27.0°C) was observed in both of the flood duration treatments and Control, the lowest temperature was recorded in the Bi-Weekly units (25.1°C). However, soil temperature in the experimental units increased sharply in June-July 2016 by nearly 2 °C from 25.1 ± 0.20 °C to 27.0 ± 0.20 °C.

SOIL pH

The pH ranged from 6.9 to 7.5 during the course of the experiment. The pH of the experimental units was alkaline throughout the experiment; however, the Control units remained slightly acidic.

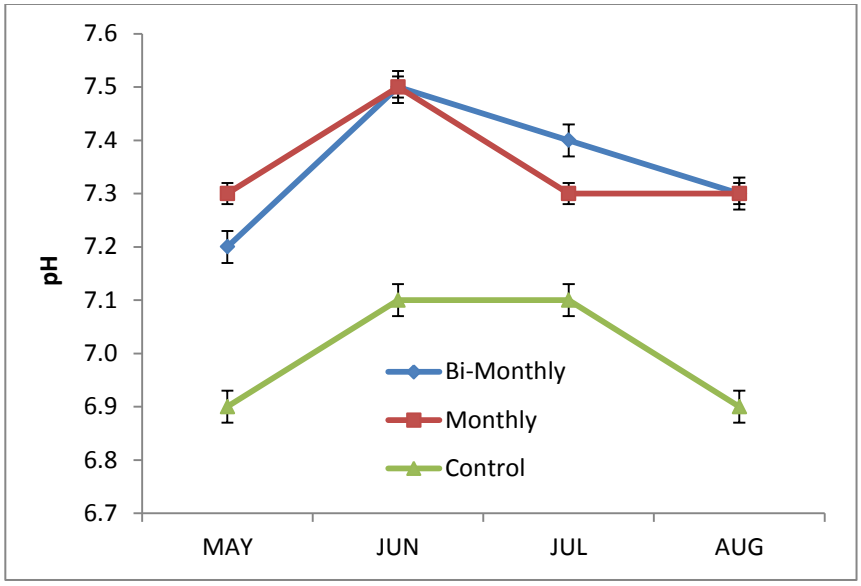


Fig. D2 The monthly mean pH for the flood duration experimental units and the control in the 123-day nutrient addition-atrazine-flood duration interaction experiment. There were no significant differences in pH between the treatments or control ($p > 0.05$)

The mean pH for all three atrazine treatments and the Control was 7.1 (Fig. D2, Table 6.1). A Student's t-test found significant differences in soil pH between the two flood duration treatments and the Control ($p < 0.0001$); however, there was no significant difference between the two flood duration treatments.

REDOX POTENTIAL

The redox potentials of the experimental and control units remained in a range above zero throughout the experiment. The range of the redox potential was +2.7 ± 19.9 mV to +166.1 ± 20.8 mV. The experimental unit and Control redox potentials fluctuated over 100 mV from June to July 2016. A Student's t-test revealed no significant differences in the soil redox potential among the two flood duration treatments and Control (Table 6.1, Fig. D3; $p < 0.0001$).

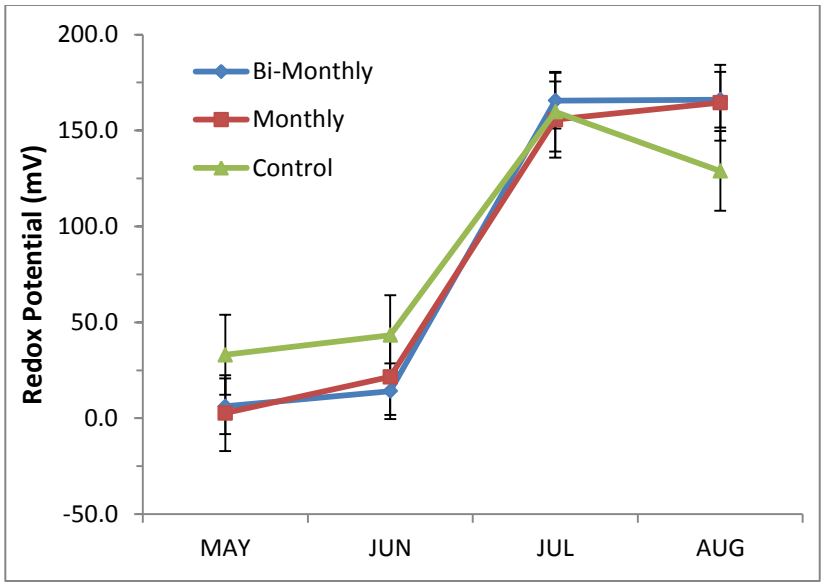


Fig. D3 The monthly mean redox potential for the flood duration experimental units and the control in the 123-day nutrient addition-atrazine-flood duration interaction experiment. There were no significant differences in redox potential between the treatments or control ($p > 0.05$)

VITA

Lauris Hollis is a native Texan and a product of the Neches River Basin in East Texas. His home, which was located at an historical nexus of longleaf savanna, coastal prairie, bottomland hardwood forests, and cypress-tupelo forested wetlands; was the impetus for his interest in wetlands ecology. Lauris is also a veteran of the United States Army where he served in the 28th Regiment, 1st Infantry Division. After military service, Lauris returned to Texas where he was employed for several years as a GIS Analyst in the Houston-Galveston region. He later enrolled at Stephen F. Austin State University, where he designed his own ecological curriculum in the College of Applied Arts and Sciences, from which he earned his Bachelor of Applied Arts and Sciences with a concentration in Natural Resources Management. He continued his ecological studies in the SFA College of Forestry and Agriculture where he earned a Master of Science in Environmental Science. In 2013, he enrolled as a PhD student in the College of Oceanography and Coastal Sciences at Louisiana State University. In May 2018, Lauris will be a candidate for the degree of Doctor of Philosophy, Oceanography and Coastal Sciences with a minor in Wetland Science and Management.